PART 1 (OF TWO PARTS)

VOLUME 71 *March, 1955* NUMBER 3

THE AMERICAN REVIEW OF Tuberculosis AND Pulmonary Diseases

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

CONTENTS

Studies on the Impairment of Respiratory Function in Pulmonary Tuberculosis. HIRATO ASSO AND JOSEPH F. TOMASHEFSKI 333

Pulmonary Resection for Tuberculosis. Experience with 200 Consecutive Patients. KENNETH CHIACHE SZE, A. SAMADI, AND JAMES S. CONANT 349

A Microcolonial Test for the Recognition of Virulent Mycobacteria. IRVING KRASNOW, LAWRENCE G. WAYNE, AND DAVID SALKIN 361

The Detection of Tubercle Bacilli in Mouth Wash Specimens by the Use of Membrane Filter Cultures. DAVID E. ROGERS, GEORGE M. COOKE, AND CHARLES E. MEYERS 371

Charcoal Agar Media for the Cultivation of *Mycobacterium Tuberculosis*. JOSEPH W. WHALEN AND W. L. MALLMANN 382

Observations on the Pathogenicity of Isoniazid-Resistant Mutants of Tubercle Bacilli for Tuberculous Patients. R. OESTERICHER, S. H. DRESSLER, W. F. RUSSELL, JR., J. B. GROW, AND G. MIDDLEBROOK 390

Methods of Finding Tuberculosis Cases and Reactors. E. J. ANDERSON 406

Investigation of Irregular Discharge of Tuberculous Patients. A Preliminary Study. THOMAS H. LORENZ, WILLIAM C. LEWIS, GEORGE CALDEN, AND JOHN R. THURSTON 419

Case Reports:

Papillomatosis of the Trachea and Bronchi. BURNETT SCHAFF AND ROBERT V. THOMSON 429

Total Tuberculous Calcification of a Kidney and Ureter. LEWIS G. JACOBS 437

Editorial: Implications of the Phenomenon of Open Cavity Healing for the Chemotherapy of Pulmonary Tuberculosis. W. F. RUSSELL, JR., S. H. DRESSLER, G. MIDDLEBROOK AND J. DEBIST 441

Notes:

Drug Susceptibilities of *M. Tuberculosis* on Charcoal Agar Medium. EDWARD LEWIN AND JAMES G. HIRSCH 447

Comparison of Roentgenographic and Surgical Findings in Tuberculosis. CHARLES E. SCHMIDT 452

The Effect of Hi Intensity Ultraviolet for Sterilization. GLADYS L. HOBBY AND TULITA F. LENERT 457

Note on the Secondary Factors Involved in the Etiology of Sarcoidosis. JOHN S. CHAPMAN 459

Books 462

American Trudeau Society: Notices 464

Abstracts 65

ANNUAL MEETING

MILWAUKEE, WIS. — MAY 23–27, 1955

Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland, by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N. Y.

Copyright 1955, by the National Tuberculosis Association

PRINTED IN U. S. A.

THE AMERICAN REVIEW OF

Tuberculosis

AND

Pulmonary Diseases

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY

Medical Section of National Tuberculosis Association

EDITOR

WALSH McDERMOTT

New York, N. Y.

CONSULTING EDITOR

ESMOND R. LONG

Philadelphia, Pa.

EDITOR OF ABSTRACTS

AARON D. CHAVES

New York, N. Y.

Helen W. Miller, *Editorial Secretary*

EDITORIAL BOARD

J. Burns Amberson, M.D., New York, N. Y.
Oscar Auerbach, M.D., East Orange, N. J.
Byron F. Francis, M.D., Seattle, Wash.
James G. Hirsch, M.D., New York, N. Y.
Kirby S. Howlett, Jr., M.D., Boston, Conn.
Daniel E. Jenkins, M.D., Houston, Tex.
Donald L. Paulson, M.D., Dallas, Tex.
Sidney Raffel, M.D., Stanford, Calif.
Philip E. Sartwell, M.D., Baltimore, Md.
John W. Strieder, M.D., Brookline, Mass.
Ralph Tompsett, M.D., New York, N. Y.
William B. Tucker, M.D., Durham, N. C.

BUSINESS MANAGER

HENRY W. STEVENS

NOTICE TO SUBSCRIBERS AND CONTRIBUTORS

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES is published by the National Tuberculosis Association and issued monthly about the first of the month. The REVIEW is the official journal of the American Trudeau Society. A volume includes six numbers and begins with the January and July numbers.

Subscription: The subscription price of the REVIEW is $12 for the calendar year. Checks should be made payable to Collier Platt, Treasurer. The price of single copies is $1.50 postpaid.

Character of the Review: The REVIEW invites the submission of original manuscripts on any phase of tuberculosis, nontuberculous pulmonary diseases, and related subjects of interest.

MANUSCRIPTS

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

Manuscripts should be sent in duplicate to the office of the Editor, Dr. Walsh McDermott, 1790 Broadway, New York 19, N. Y., mailed flat, and transmitted by first-class mail with postage for return if not acceptable. They should be in English, double-spaced, typewritten on one side of the page with wide margins. Tabular data should not be included in the text but should be presented in separate tables with appropriate reference in the text. All cases of pulmonary tuberculosis should be classified according to the 1950 edition of *Diagnostic Standards and Classification of Tuberculosis* published by the National Tuberculosis Association. All case reports should be written in complete sentences. Authors should exercise particular care in the preparation, notation, and description of figures and tables. The form of reference used in the REVIEW should be carefully followed and references should be checked for accuracy. Each table must be accompanied by a descriptive title and a suitable legend must be provided for each figure. Drawings must be made with india ink and all lettering should be made with a stencil. The quality of the reproduction of a roentgenogram depends upon both the quality of the original picture and of the glossy print obtained from photographing that picture. If a contributor does not have facilities available, preparation of figures or reproduction of roentgenograms may be handled through the REVIEW office. In those circumstances, the contributor should supply a neat, properly proportioned, and suitably entitled drawing or the original roentgenogram, and will be expected to defray the cost of these services.

The publishers will not be responsible for manuscripts, illustrations, etc., lost in transit. In order to save expense for authors' corrections in proof, manuscripts should be carefully revised by the author before submission.

Reprints: Fifty reprints without covers of articles will be furnished to authors free of charge when requested in advance. Additional reprints may be ordered.

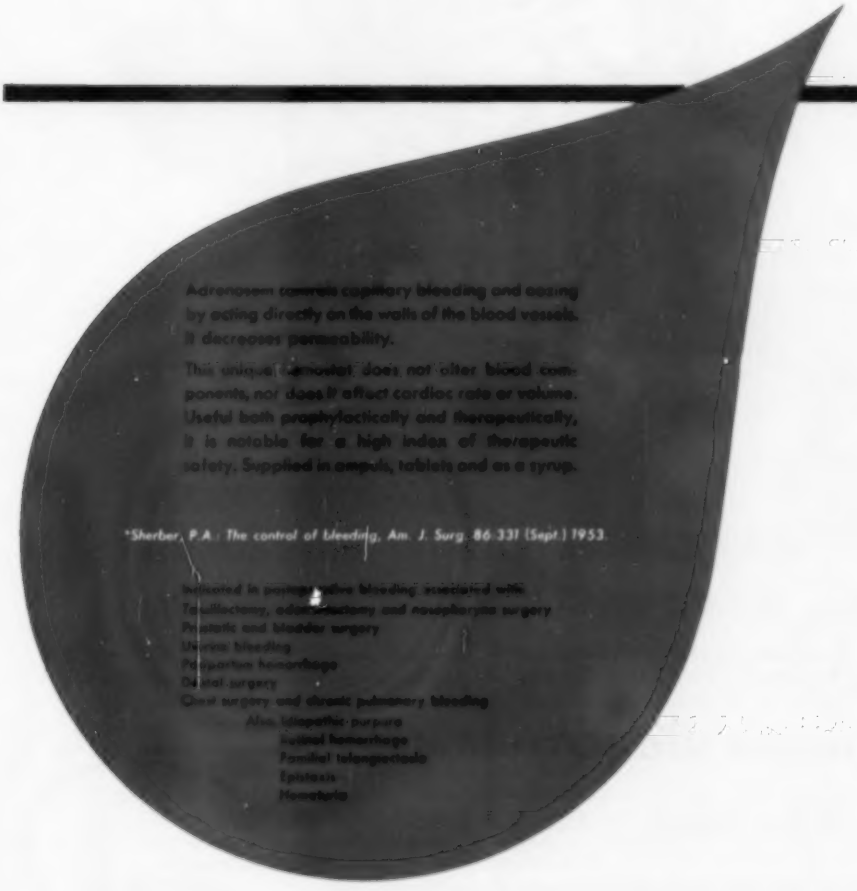
Advertising: Rates will be furnished by THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES, 1790 Broadway, New York 19, N. Y. on request.

The Publishers reserve the right to decline any advertising submitted. Acceptance of an advertisement does not constitute official endorsement of the product advertised.

Entered as second-class matter March 9, 1917, at the post office at Baltimore, Md., under the Act of August 24, 1912. Acceptance for mailing at a special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 2, 1918.

"...a potent antihemorrhagic factor"*

in use in more than 2500 hospitals


Adrenosem controls capillary bleeding and oozing by acting directly on the walls of the blood vessels. It decreases permeability.
This unique hemostat does not alter blood components, nor does it affect cardiac rate or volume. Useful both prophylactically and therapeutically, it is notable for a high index of therapeutic safety. Supplied in ampuls, tablets and as a syrup.
*Sherber, P.A.: The control of bleeding, Am. J. Surg. 86:331 (Sept.) 1953.
Indicated in postoperative bleeding associated with:
Tonsillectomy, adenoidectomy and nasopharynx surgery
Prostatic and bladder surgery
Uterine bleeding
Postpartum hemorrhage
Dental surgery
Chest surgery and chronic pulmonary bleeding
Also Idiopathic purpura
Scleral hemorrhage
Familial telangiectasia
Epistaxis
Hematuria

The unique systemic hemostat

Send for detailed literature

Adrenosem®

SALICYLATE

(BRAND OF CARBAZOCHROME SALICYLATE)

THE S. E. MASSENGILL COMPANY, Bristol, Tennessee

RAYTHEON MICROTHERM *advantages when*

Pulmonary TB is present

Raytheon Microtherm® Diathermy makes it possible to deliver deep heat to the tuberculosis patient almost as safely as to the non-tubercular.[1] No harmful effects are observed as far as the pulmonary lesion is concerned.[1] Marked benefits result in the treatment of osteo-arthritis, fibromyositis, bursitis, peripheral neuritis and sprains.[1]

Areas at a distance from the lungs—ankle, knee, and hip joints, and the sciatic nerve distribution can be treated without extra precautions, by using the directors best suited to the particular area and condition.[1]

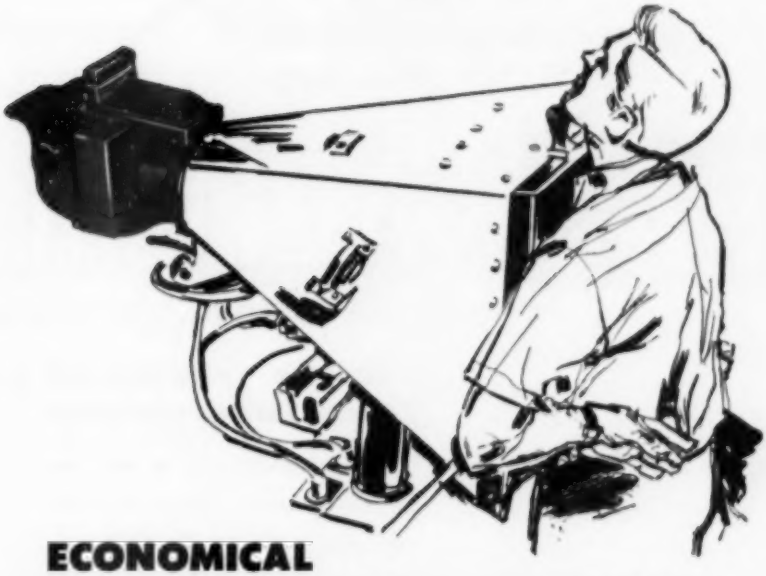
Your medical equipment dealer will gladly demonstrate new Raytheon Microtherm, Model CMD-10.

1. *Rudin, L. N., and Jensen, J. R., C. M. Digest, XX: 7, 1953*
Copies of this article are available on request.

RAYTHEON

Excellence in Electronics

RAYTHEON MANUFACTURING COMPANY
Microwave and Power Tube Operations, Section NB-TA, Waltham 57, Mass.



ECONOMICAL
ADMISSION X-RAY PROGRAMS
made possible by Fairchild 70-mm cameras

Fairchild 70-mm x-ray cameras, used in connection with photofluorographic equipment, provide the easiest and most economical method of carrying out a complete admissions x-ray program – because of their rapid, automatic operation and fractional film costs. As a result, these cameras have become the "standard" for mass chest radiography. The 70-mm negative is adequate for direct viewing; magnification viewing is available if desired. Suspected positive cases (which have been found to average between 8 and 10 per cent of all hospital admissions) would normally be retaken on 14 x 17 film by the hospital radiologist.

The completely automatic operation of the Fairchild Roll Film Camera permits one technician to radiograph up to 150 chests per hour. For smaller hospitals the Cut Film Camera offers identical high negative quality at lower initial investment. Fairchild's 70-mm cameras are available on all leading 70-mm hospital admission units and can be adapted to many existing installations. The cameras are unconditionally guaranteed for one year, and are backed by Fairchild factory service. For further information consult your x-ray equipment supplier or write *Fairchild Camera and Instrument Corp., 88-06 Van Wyck Expressway, Jamaica, N. Y., Dept. 160-3583.*

Report of the Council of Tuberculosis Committees, American College of Chest Physicians, April, 1951:

". . . We also urge routine chest x rays of all admissions to general hospitals. The value of this has been demonstrated in several large hospitals throughout the country where unrecognized cases of tuberculosis and other chest conditions are being discovered."

FAIRCHILD
X-RAY EQUIPMENT
AND ACCESSORIES

The FIRST real improvement in P.A.S. therapy

PASKALIUM

(POTASSIUM PARA-AMINOSALICYLATE)*

...in the treatment of tuberculosis

AVOIDS

THESE COMMON SIDE REACTIONS . . .

ANOREXIA
NAUSEA
VOMITING
DIARRHEA

Well tolerated, even by most patients with known histories of intolerance to all previous P.A.S. therapy. Wherever Para-Aminosalicylic Acid is to be used, PASKALIUM will give the best results.

Available now:

PASKALIUM powder,
100 gm., 1 lb., 5 lbs.
Bulk units 25 lbs.
"Envules"
3 grams each.

- HIGHER P.A.S. BLOOD LEVELS
- BETTER TOLERATED
- PREFERRED BY PATIENTS

Please request physician's literature

GL

GLENWOOD LABORATORIES
INCORPORATED

Ethical Drug Manufacturers

TEANECK, N. J.

*U.S. PATENT PENDING

General Electric

announces the REGENT

a moderately priced, highly versatile x-ray unit

Featuring . . .

a compact, lightweight spot-film device and maneuverable, space-saving overhead tube support

For fluoroscopy . . . for radiography — General Electric's new REGENT offers radiologists outstanding facilities. New ease and convenience is found in the compact spot-film device. One of the lightest ever offered, it also assures fast, simple operation . . . accepts either 10 x 12 or 8 x 10 cassettes . . . provides eight field sizes. It's available with or without phototiming.

For radiography the REGENT offers an overhead tube support that's instantly positioned for all technics — including all cross-table, decubitus or hospital-cart radiography. Provides increased working space around the table. Easily parked out of the way when not in use. Also available is a conventional tube stand that can be floor-to-ceiling or platform mounted.

Equally important — with the REGENT you get a product backed by the prestige of General Electric . . . in keeping with your own fine standards. Research, engineering, manufacture, service — all are performed with the skill and care you naturally associate with General Electric.

Ask your G-E x-ray representative to show you how the REGENT can add ease and facility to your radiography and fluoroscopy. Or write X-Ray Department, General Electric Company, Milwaukee 1, Wisconsin for Pub. 531.

45° TRENDELENBURG fulfills requirements for virtually all technics. Hydraulic tilting mechanism is foot-pedal controlled — moves table at any speed you prefer.

REGENT utilizes Scholz lightweight duplex spot-film device with Scholz optical phototiming pickup or sealed G-E unit as pictured.

Progress Is Our Most Important Product

GENERAL ELECTRIC

A NEW COUGH SPECIFIC

Free from central depression

NON-NARCOTIC

Free from addiction

TESTED IN 18,000 OBSERVATIONS*

No constipation

Romilar / 'Roche'

a 10-mg dose of Romilar

is equivalent to

a 15-mg dose of codeine

available in tablets

and as a syrup

*L. J. Cass et al., *New England J. Med.*, 249:132, 1953; *Am. J. M. Sc.*, 227:291, 1954.

Romilar® Hydrobromide – brand of

dextromethorphan hydrobromide

(d-3-methoxy-N-methylmorphinan hydrobromide)

HOFFMANN-LA ROCHE INC

Roche Park • Nutley 10 • New Jersey

THE AMERICAN REVIEW OF
Tuberculosis
AND
Pulmonary Diseases

VOLUME 71 *March, 1955* NUMBER 3

STUDIES ON THE IMPAIRMENT OF RESPIRATORY FUNCTION IN PULMONARY TUBERCULOSIS[1]

HISATO ANNO AND JOSEPH F. TOMASHEFSKI

(Received for publication July 1, 1954)

INTRODUCTION

Since Riley and his associates (1, 2) developed a method for the evaluation of ventilation-perfusion relationships and oxygen-diffusing capacity of the lung, several reports have appeared relating the impairment of these functions in pulmonary emphysema, pulmonary fibrosis, Boeck's sarcoid, and mitral stenosis (3–9). McClement and associates (10) reported on miliary tuberculosis, and Anderson, Bell, and Blount (11) included a few cases of tuberculosis in a general report on chronic pulmonary diseases.

The object of the present study is to determine the types of respiratory impairment which occur in pulmonary tuberculosis and to compare this impairment with the nature and extent of the disease process. In several patients an attempt will be made to evaluate the effect of chemotherapy and surgery on the ventilation-perfusion relationships.

MATERIAL AND METHODS

Twenty-five selected patients with proved pulmonary tuberculosis, without evidence of cardiac disease, representing various types and degrees of involvement were studied. In accordance with the 1950 National Tuberculosis Association classification, made at the time of the first study, 8 patients were classified as having far advanced; 8, moderately advanced; and 3, minimal pulmonary tuberculosis. Also included were 2 cases of miliary tuberculosis, and one case each of unexpandable lung, fibrothorax, pleurisy with effusion, and peritracheal tuberculous lymphadenitis. Follow-up studies were made on 9 of the patients after a period of chemotherapy and/or surgery.

Vital capacity, three-second timed vital capacity, and maximal breathing capacity (MBC) were determined on a modified Benedict-Roth spirometer of 13.5 liters capacity. Vital capacity was predicted by the formulas derived by Baldwin and her associates (12),

[1] From the Cardio-Respiratory Laboratory, Ohio Tuberculosis Hospital; and the Department of Medicine, Ohio State University College of Medicine, Columbus, Ohio.

whereas maximal breathing capacity was predicted by the formulas of Motley (13). Residual volume was determined, except for slight modification, by the method of Darling and co-workers (14). Predicted residual volume and total lung capacity were derived from the formulas of Kaltreider, Fray, and Hyde (15). The partial pressures of oxygen and carbon dioxide in the arterial blood were determined by the method of Riley, Proemmel, and Franke (16). A microscopic stage was used to facilitate measuring the bubble size. Correction factors recommended by Riley were not found to be necessary. Arterial blood pH was determined at 37°C. with a Cambridge research model pH meter equipped with a constant-temperature water jacket. The same arterial blood was analyzed for oxygen saturation and carbon dioxide content by the Van Slyke-Neill manometric method (17).

The arterial blood oxygen saturation obtained from the oxyhemoglobin dissociation curve, using the directly determined pH and P_{O_2}, checked within plus-minus 1 per cent at the high level of oxygenation and within plus-minus 2 per cent at the low level of oxygenation with the saturation obtained by the Van Slyke method. The P_{CO_2} directly determined checked within plus-minus 2 mm. of mercury with the P_{CO_2} obtained indirectly by the method of Van Slyke and Sendroy (18) using the oxygen capacity, pH, and carbon dioxide content.

Determination and analysis of the alveolar-arterial oxygen pressure gradient was made in the manner outlined by Riley and his associates (1, 2). For the calculation of the oxygen diffusion capacity and per cent venous admixture a difference in saturation between the pulmonary capillary and mixed venous blood of 25 per cent was assumed. When the oxygen diffusion capacity was more than 9 cc. per minute per mm. of mercury per square meter of body surface area or the diffusion gradient at the low oxygen level was less than 6 mm. of mercury, it was classified as normal (8).

RESULTS

The age of the patients, their physical characteristics, the classification of the extent of disease and its known duration, and the treatment received are presented in table 1. The results of pulmonary function testing are presented in tables 2 and 3, in which standardized symbols are used (19). The patients with uncomplicated parenchymal pulmonary tuberculosis, excluding miliary tuberculosis, are arranged according to the National Tuberculosis Association classification.

Far Advanced Pulmonary Tuberculosis

There were 8 patients with far advanced pulmonary tuberculosis. Four (*Cases 1* to *4*) showed a moderate to marked impairment of ventilatory function. Five (*Cases 1* to *5*) showed an abnormally high residual volume to total lung capacity ratio (RV/TLC). In 7 patients the index of intrapulmonary gas mixing was more than 2 per cent. If 30 per cent is considered as the upper limit of normal dead-space ventilation and 6 per cent as the upper limit of normal venous admixture, respectively, then 6 patients in this group showed evidence of increased dead-space ventilation and 7 patients showed evidence of increased venous admixture.

Impairment of the oxygen diffusion capacity of the alveolar-capillary membrane was detected in 5 patients; 4 of these 5 were in the group having an increase in the RV/TLC ratio.

One patient in the group showed no evidence of either impairment of ventilation, perfusion, or diffusion, but did have an increased RV/TLC ratio (*Case 5*). Another patient (*Case 8*) showed only a slight reduction in ventilatory function, with normal residual volume and normal distribution and diffusion.

TABLE 1

PHYSICAL CHARACTERISTICS, CLASSIFICATION, AND CLINICAL DATA IN 25 CASES OF PULMONARY TUBERCULOSIS

Number	Name	Sex	Age	Body Surface Area m²	Classification (NTA)		Duration* of Disease	Duration† of Chemotherapy
1	L. B.	M	40	1.66	Far advanced		7 years	6 months
2	D. L.	M	52	1.89	Far advanced		4 years	6 months
3	D. G.	M	46	1.59	Far advanced		12 years	6 months
4	W. B.	M	43	1.84	Far advanced	*a*)	1 month	10 days
				1.89		*b*)		4 months
5	D. D.	M	41	1.84	Far advanced		7 years	6 months
6	E. H.	M	21	1.81	Far advanced		4 years	3 months
7	E. K.	M	30	1.79	Far advanced		5 months	none
8	C. H.	M	19	1.74	Far advanced	*a*)	3 months	3 months
				1.78		*b*)		5 months + Pnpm.‡
9	J. H.	M	51	1.46	Moderately advanced		6 months	2 months
10	L. F.	M	52	1.76	Moderately advanced	*a*)	5 months	2 months
				1.81		*b*)		4 months
11	V. R.	M	25	1.88	Moderately advanced	*a*)	7 months	4 weeks
				1.90		*b*)		4 months
12	L. G.	F	24	1.56	Moderately advanced		7 months	1 week
13	R. A.	M	23	1.79	Moderately advanced	*a*)	2 months	2 weeks
				1.84		*b*)		3 months
14	M. E.	M	33	1.80	Moderately advanced		1 month	2 weeks
15	J. P.	M	55	1.68	Moderately advanced		6 months	6 weeks
16	G. D.	M	40	1.60	Moderately advanced	*a*)	2 years	none
				1.67		*b*)		2 months + Pnpm.‡
17	W. G.	M	18	1.73	Minimal		3 months	none
18	B. D.	F	24	1.60	Minimal		2 months	8 weeks
19	J. B.	M	31	1.88	Minimal		6 months	7 weeks
20	J. T.	F	18	1.47	Miliary	*a*)	3 weeks	6 days
				1.52		*b*)		3 months
21	W. L.	F	29	1.71	Miliary		7 weeks	3 weeks
22	F. G.	F	40	1.60	Unexpandable lung	*a*)	14 years	none
				1.60		*b*)		2 months postoperatively
23	V. L.	F	31	1.53	Fibrothorax	*a*)	12 years	1 week
				1.52		*b*)		2 months postoperatively
24	D. V.	F	24	1.66	Moderately advanced with effusion		7 months	6 days
25	H. S.	M	24	1.77	Peritracheal lymphadenopathy		7 months	none

* From the onset of symptoms or the first positive roentgenogram.

† From the start of drug therapy to time of study: *a*. initial study; *b*. follow-up study.

‡ Pnpm. = Pneumoperitoneum.

TABLE 2

VITAL CAPACITY, MAXIMAL BREATHING CAPACITY, RESIDUAL VOLUME, AND TOTAL LUNG CAPACITY IN 25 CASES OF PULMONARY TUBERCULOSIS

Number	Name	VC		Timed VC Per Cent 3 Seconds	MBC		RV		TLC		RV/TLC	IIM†
		Observed Values	Per Cent*		Observed Values	Per Cent*	Observed Values	Per Cent*	Observed Values	Per Cent*	Per Cent	Per Cent
Normal		—	100	95	—	100	—	100	—	100	<35	<2
1	L. B.	2,555	60	76	42	30	3,289	249	5,394	96	61	4.9
2	D. L.	3,499	91	71	54	40	4,509	264	7,699	139	59	3.2
3	D. G.	2,576	66	81	73	63	1,828	154	4,148	81	44	5.5
4	W. B.	3,960	96	90	98	71	2,424	190	6,364	117	38	2.1
5	D. D.	4,170	100	100	154	109	3,058	239	7,216	134	42	2.5
6	E. H.	4,280	95	96	149	95	1,561	137	5,619	99	28	2.4
7	E. K.	4,962	120	96	172	117	1,988	192	6,688	130	30	2.2
8	C. H.	3,805	84	98	138	90	1,168	104	4,674	83	25	1.5
9	J. H.	3,420	98	91	78	75	1,985	127	5,325	105	37	2.0
10	L. F.	4,920	129	90	145	116	889	52	5,689	103	16	1.1
11	V. R.	5,915	131	93	164	103	1,395	124	7,275	129	19	1.1
12	L. G.	2,890	89	100	135	122	1,273	156	4,243	104	30	1.5
13	R. A.	5,950	132	98	168	110	1,655	147	7,525	136	22	1.4
14	M. E.	4,460	107	99	167	115	1,619	156	5,919	114	27	1.5
15	J. P.	3,570	101	89	81	70	1,547	98	6,113	100	30	1.2
16	G. D.	3,680	92	98	119	96	1,276	104	4,576	87	28	1.8
17	W. G.	4,435	98	100	149	98	1,254	111	5,689	101	22	1.0
18	B. D.	3,938	124	100	111	98	1,341	170	5,281	134	25	1.2
19	J. B.	4,690	109	100	174	114	1,457	134	6,027	111	22	1.4
20	J. T. *a*)	2,622	82	98	93	86	789	98	3,329	81	24	0.6
	b)	2,830	88	99	131	117	791	98	3,468	87	23	1.0
21	W. L.	3,186	105	97	94	80	1,016	134	3,984	105	26	1.5
22	F. B. *a*)	2,495	93	97	64	63	760	93	3,190	89	24	1.8
	b)	2,154	81	91	67	66	1,145	140	3,027	87	38	1.2
23	V. L. *a*)	2,038	71	95	91	83	684	95	2,654	74	26	1.1
	b)	1,570	55	94	59	58	869	121	2,304	65	38	1.1
24	D. V.	2,485	78	97	88	77	735	92	2,795	71	26	0.8
25	H. S.	3,008	72	83	116	77	1,799	171	4,829	92	37	1.8

* Per cent of predicted normal value.

† IIM = Index of intrapulmonary gas mixing, alveolar nitrogen after seven minutes of oxygen breathing: *a*. initial study; *b*. follow-up studies.

The following case report is given as a typical example of impairment of respiration found in far advanced pulmonary tuberculosis.

Case 3. D. G., a 46-year-old Negro, was admitted to the Ohio Tuberculosis Hospital on January 12, 1953. His tuberculosis dated back to 1942 when he was rejected from military service because of roentgenographic findings. From September, 1942, until March, 1943, he was hospitalized in a tuberculosis sanatorium. He felt well until the summer of 1952, at which time he noticed weakness, weight loss, productive cough, and shortness of breath on exertion.

On admission the chest roentgenogram revealed multiple cavitation surrounded by mottled densities in both upper lung fields, more extensive on the left. There was blunting of the right costophrenic angle. A slight scoliosis of the dorsal spine was noted.

Sputum was positive for acid-fast bacilli on microscopy. The patient was treated with streptomycin and para-aminosalicylic acid (PAS), with remarkable clinical improvement. However, the roentgenogram of the chest at the time of the study (figure 1) showed persistent bilateral cavitary disease with a slight diminution in the size of the cavities on the left and some clearing of the disease in the right upper lobe.

Physiologic evaluation of this patient revealed impairment of ventilation, increase in residual volume, dead-space ventilation, venous admixture, and a decrease in oxygen diffusion capacity of the alveolar-capillary membrane.

Moderately Advanced Pulmonary Tuberculosis

Of the 8 patients studied in this group, 2 showed an impairment of ventilation; one, an increase in the RV/TLC ratio; 6, an increase in venous admixture; and 3, an increase in dead-space ventilation.

One patient (*Case 15*) in this group had a normal venous admixture in the presence of an increase in dead-space ventilation. In all the others, those with both moderately advanced and far advanced disease, an increase in dead-space ventilation was accompanied by either an increase in venous admixture alone or an increase in venous admixture and a decrease in the oxygen diffusion capacity of the alveolar-capillary membrane.

The following case report is presented in detail to illustrate an impairment in respiratory gas exchange in the presence of normal ventilatory function and a normal residual volume.

Case 10. L. F., a 52-year-old white male, was admitted to the Ohio Tuberculosis Hospital on December 21, 1952. Symptoms consisting of a productive cough, night sweats, and weight loss were first noticed in July, 1953. A survey film taken in August, 1953, revealed disease in the right apex. Sputum was positive for tubercle bacilli.

On admission, when the first study was performed, the chest roentgenogram (figure 2) showed a partially shrunken right upper lobe in which some dense irregular areas of infiltration and a small cavity were present. The lower border of the diseased area was sharply delineated by the minor fissure. The left lung field was clear.

This patient was studied on two occasions, one week after the start of streptomycin-PAS therapy and again four months after chemotherapy was given. A chest roentgenogram taken at the time of the second study showed some clearing of the disease in the right upper lobe with closure of the cavity. The left lung was still uninvolved.

Physiologic evaluation of this case revealed normal ventilatory measurements, a normal residual volume, a slight increase in dead-space ventilation, a marked increase in venous admixture, and a moderate impairment of the oxygen diffusion capacity. After four months of chemotherapy, follow-up studies revealed these findings to be essentially unchanged in spite of roentgenographic evidence of improvement.

Minimal Pulmonary Tuberculosis

Three patients with minimal tuberculosis were evaluated. Except for a very slight increase in venous admixture in one patient in whom the disease occupied the entire right supraclavicular region, all of the physiologic measurements fell within normal limits.

TABLE 3

VENTILATION, ARTERIAL BLOOD, DISTRIBUTION, AND DIFFUSION FINDINGS IN 25 CASES OF PULMONARY TUBERCULOSIS*

Number	Name	$P_{I_{O_2}}$ mm. Hg	$P_{eA_{O_2}}$ mm. Hg	$P_{a_{O_2}}$ mm. Hg	A-a Gradient mm. Hg	P_{aco_2} mm. Hg	$P_{E_{CO_2}}$ mm. Hg	$\dot{V}_E$ L/min/m² BTPS	f	$\dot{V}_{O_2}$ cc/min/m² STPD	R	$\frac{V^s_D}{V_T}$ per cent	$\frac{\dot{Q}_{V_S}}{\dot{Q}_T}$ per cent	D_{O_2}/m²	Sa_{O_2}
1	L. B.	191	137	68	69	44	19	6.17	23	176	0.757	43	30	3	84
		146	95	45	50	42	18	6.50	24	172	0.781	44			
2	D. L.	146	100	73	27	38	20	4.97	15	125	0.857	38	16	5	94
		117	74	55	19	38	19	4.67	17	120	0.853	38			
3	D. G.	147	105	77	28	39	17	5.83	24	124	0.904	41	15	5	94
		120	80	61	20	37	14	6.47	27	118	0.911	46			
4	W. B. *a)*	145	104	58	46	38	20	6.24	17	157	0.913	39	36	6	90
		111	71	43	28	37	20	5.82	17	149	0.921	37			
	b)	148	101	59	42	39	21	4.79	15	146	0.790	36	34	9	89
		98	52	41	11	40	22	4.62	14	133	0.855	35			
5	D. D.	146	98	86	12	41	24	4.07	16	133	0.830	29	6	9	95
		96	54	45	9	39	25	4.12	15	132	0.876	24			
6	E. H.	149	101	83	18	40	21	4.65	19	142	0.795	34	10	8	95
		97	60	47	13	35	19	5.60	26	127	0.946	30			
7	E. K.	149	97	82	15	42	31	3.32	10	153	0.771	17	9	12	95
		98	53	50	3	40	30	4.16	9	161	0.877	18			
8	C. H. *a)*	143	95	86	9	41	27	3.93	12	151	0.812	24	5	12	96
		95	50	41	9	39	28	3.92	11	150	0.842	20			
	b)	145	98	87	11	39	27	3.68	12	141	0.797	20	6	10	97
		97	54	47	7	38	29	3.82	10	146	0.861	15			
9	J. H.	146	98	75	23	42	20	4.86	19	128	0.858	34	14	14	94
		112	65	55	10	40	19	4.81	20	115	0.837	35			
10	L. F. *a)*	144	107	74	33	32	20	6.99	22	164	0.938	32	16	6	94
		111	75	54	21	35	19	6.93	23	159	0.954	34			
	b)	147	106	76	30	38	22	5.21	17	145	0.898	31	16	6	95
		120	81	61	20	37	22	5.47	18	148	0.924	30			

11	V. R. *a*)	143	114	94	20	31	23	5.26	13	129	1.068	18	8	6	97
		110	73	61	12	34	24	3.54	12	112	0.893	19			
	b)	146	109	89	20	34	24	3.84	13	119	0.891	19	8	7	97
		97	59	50	9	34	25	4.00	13	120	0.878	16			
12	L. G.	145	103	88	15	34	21	3.62	17	113	0.781	20	7	18	96
		95	62	59	3	32	22	4.18	16	109	0.976	17			
13	R. A. *a*)	144	100	84	16	38	27	3.38	8	125	0.842	21	8	11	96
		96	54	48	6	39	27	3.74	11	126	0.922	21			
	b)	146	100	83	17	42	28	3.75	13	136	0.879	22	9	9	95
		97	53	46	7	41	26	4.36	15	141	0.928	25			
14	M. E.	146	99	80	19	43	24	4.35	18	131	0.892	30	10	8	95
		97	54	52	2	40	23	4.60	18	128	0.935	30			
15	J. P.	147	115	102	13	33	18	6.60	27	126	1.043	31	4	9	98
		113	77	70	7	33	17	6.13	26	130	0.895	33			
16	G. D. *a*)	147	97	86	11	39	24	4.59	11	173	0.735	30	6	7	95
		97	53	48	5	36	22	5.03	13	160	0.793	29			
	b)	146	97	81	16	38	25	3.92	11	156	0.730	24	8	10	95
		97	55	46	9	36	23	4.48	12	142	0.849	26			
17	W. G.	148	104	89	15	42	27	3.94	14	132	0.931	23	6	13	96
		97	54	51	3	41	26	4.29	14	127	0.936	24			
18	B. D.	146	102	89	13	38	27	3.40	13	127	0.836	15	6	9	96
		96	55	48	7	38	26	3.93	14	127	0.905	18			
19	J. B.	144	102	94	8	38	26	4.16	11	138	0.886	23	4	7	98
		111	69	61	8	36	24	4.52	12	151	0.845	25			
20	J. T. *a*)	147	104	83	21	36	20	6.18	27	170	0.800	27	10	7	96
		113	75	60	15	33	20	6.47	27	174	0.838	22			
	b)	143	101	88	13	35	23	4.32	21	140	0.801	15	6	8	95
		95	58	48	10	32	22	5.06	21	146	0.836	14			
21	W. L.	144	106	80	26	36	23	4.48	18	128	0.922	22	13	9	96
		98	61	51	10	32	19	5.37	27	135	0.840	23			
22	F. B. *a*)	145	108	81	27	33	20	4.94	25	125	0.866	21	15	7	95
		111	76	60	16	31	20	5.19	24	139	0.852	18			
	b)	145	99	75	24	39	25	3.90	18	141	0.805	18	14	9	94
		96	54	43	11	37	23	4.57	20	143	0.858	21			

TABLE 3—*Continued*

Number	Name	$P_{I_{O_2}}$ mm. Hg	$Pe_{A_{O_2}}$ mm. Hg	$P_{a_{O_2}}$ mm. Hg	A-a Gradient mm. Hg	$P_{a_{CO_2}}$ mm. Hg	$P_{E_{CO_2}}$ mm. Hg	$\dot{V}_E$ L/min/m² BTPS	f	$\dot{V}_{O_2}$ cc/min/m² STPD	R	$\frac{V_D}{V_T}$ per cent	$\frac{\dot{Q}_{V_a}}{\dot{Q}t}$ per cent	D_{O_2}/m^2	Sa_{O_2}
23	V. L. *a*)	146	104	77	27	37	21	4.04	20	114	0.853	24	14	8	94
		112	68	59	9	39	21	4.05	19	114	0.867	28			
	b)	146	104	88	16	40	25	3.68	15	93	0.926	18	8	8	95
		97	58	52	6	39	23	4.13	17	107	1.006	25			
24	D. V.	146	110	87	23	31	20	5.75	25	141	0.905	20	10	9	97
		96	64	55	9	29	19	5.62	23	136	0.907	20			
25	H. S.	145	100	65	35	38	27	4.14	13	154	0.811	18	25	10	90
		96	59	44	15	34	26	5.04	12	164	0.918	15			

* Duplicate figures refer to two levels of oxygen breathing: *a* initial study; *b* follow-up study.

Symbols

(in alphabetical order)

A-a = alveolar-arterial oxygen pressure gradient in millimeters of mercury
BTPS = body temperature, prevailing barometric pressure, and saturated with water vapor
D_{O_2}/m^2 = diffusion capacity of the lungs per square meter of body surface area
f = respiratory rate in breaths per minute
$Pe_{A_{O_2}}$ = "effective" alveolar P_{O_2} in millimeters of mercury
$P_{a_{O_2}}$ = arterial P_{O_2} in millimeters of mercury
$P_{a_{CO_2}}$ = arterial P_{CO_2} in millimeters of mercury
$P_{E_{CO_2}}$ = expired gas P_{CO_2} in millimeters of mercury, BTPS
$P_{I_{O_2}}$ = inspired gas in millimeters of mercury, BTPS
$\frac{\dot{Q}_{V_a}}{\dot{Q}t}$ = ratio of venous admixture to total blood flow, expressed as per cent
R = the CO_2-O_2 exchange ratio
Sa_{O_2} = arterial oxygen saturation
STPD = standard temperature, pressure, dry (0° C., 760 mm. of mercury)
$\frac{V_D}{V_T}$ = ratio of dead space to tidal volume corrected for apparatus dead space
$\dot{V}_E$ = minute ventilation
$\dot{V}_{O_2}$ = oxygen uptake in cc/min/m² body surface area

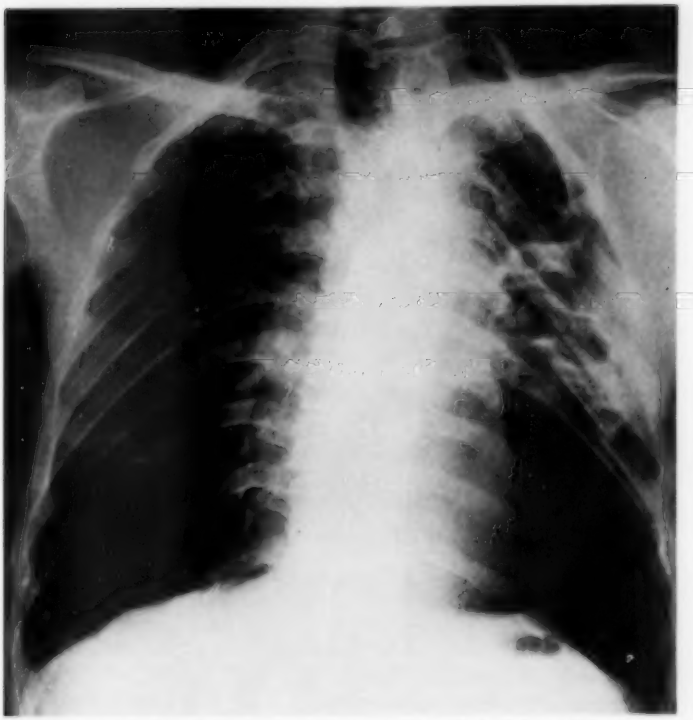


FIG. 1. *Case 3* (D. G.) Far advanced pulmonary tuberculosis with bilateral cavitary disease. Physiologic evaluation revealed impairment of ventilatory function, increase in residual volume, dead-space ventilation, and venous admixture as well as a decrease in oxygen diffusion capacity.

Miliary Tuberculosis

Both of the patients with miliary tuberculosis who were studied showed a slight reduction in the maximal breathing capacity, with normal residual volume and arterial oxygen saturation. There was, however, a significant increase in venous admixture in both patients and an impairment of oxygen diffusion in one. The latter patient was studied again when the chest roentgenogram showed complete resolution of the disease process after three months of chemotherapy. At this time the dead-space ventilation had decreased, venous admixture had returned to normal, and oxygen diffusion capacity had improved.

Miscellaneous Forms of Tuberculosis

Unexpandable lung:

Case 22. F. B. (figures 3*a*, 3*b*), a 42-year-old white female, developed an unexpandable lung during pneumothorax therapy which was initiated fourteen years before for the treatment of moderately advanced cavitary pulmonary tuberculosis. The right lung had remained

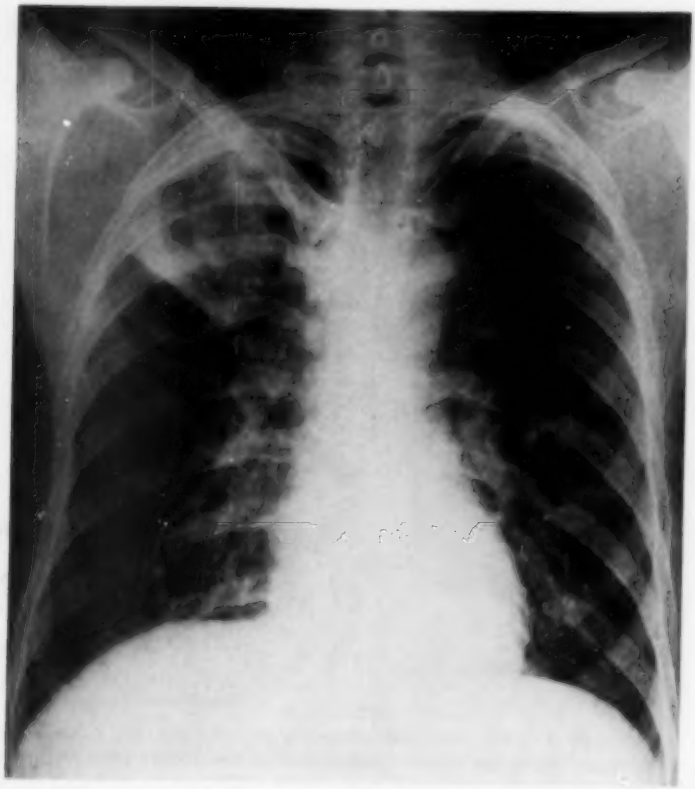


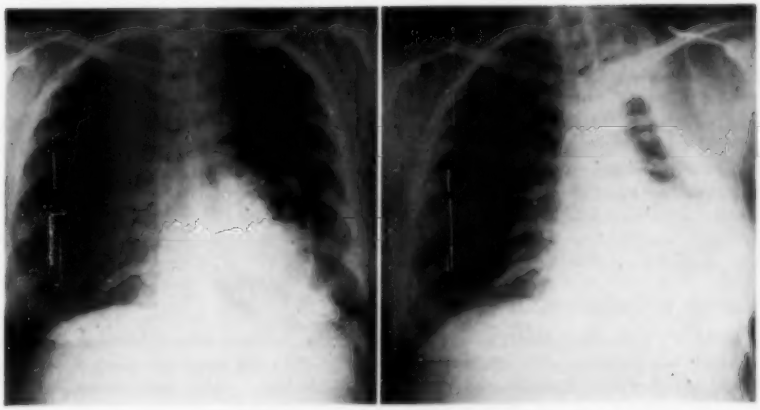
FIG. 2. *Case 10* (L. F.) Moderately advanced pulmonary tuberculosis with a partially shrunken right upper lobe. Physiologic evaluation revealed an increase in venous admixture, a slight increase in dead space ventilation, and impairment of oxygen diffusion in the presence of normal ventilatory function and a normal residual volume.

clear throughout this entire period, and there was no evidence of pleural effusion at any time. On admission a chest roentgenogram showed approximately 90 per cent collapse of the left lung. Bronchospirometry revealed the left side to be providing 20 per cent of the minute ventilation, 9 per cent of the oxygen uptake, and 9 per cent of the total vital capacity. Physiologic measurements at this time showed a decrease in the maximal breathing capacity and an increase in venous admixture.

A decortication, resection of residual foci, and a "tailoring" thoracoplasty were performed. Two months after the operation, physiologic evaluation revealed a slight decrease in vital capacity, some increase in residual volume, and essentially no change in the respiratory gas exchange measurements.

Fibrothorax:

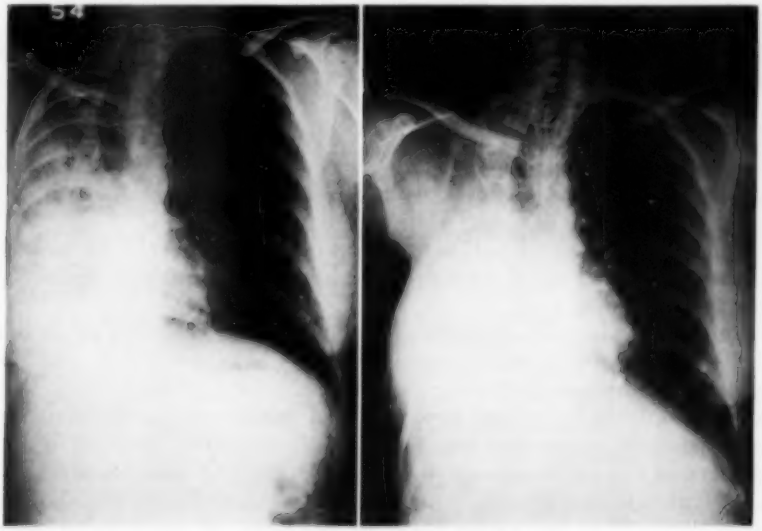
Case 23. V. L. (figures 4*a*, 4*b*), a 31-year-old white female, developed an extensive fibrothorax following four years (1942 to 1946) of pneumothorax treatment of the right lung. Roentgenographic examination of the chest at the time of the study showed a shift of the



FIGS. 3 *a* and *b*. *Case 22* (F. B.)

a. (Left) Unexpandable left lung following fourteen years of pneumothorax therapy. Physiologic evaluation revealed a reduction in the maximal breathing capacity and an increase in venous admixture.

b. (Right) Two months after a decortication, resection of residual foci of disease and a "tailoring" thoracoplasty. Physiologic evaluation at this time reveals a slight reduction in the vital capacity, an increase in the residual volume, but essentially no change in respiratory gas exchange measurements.



FIGS. 4 *a* and *b*. *Case 23* (V. L.)

a. (Left) Extensive fibrothorax of the right lung following four years of pneumothorax therapy. Physiologic evaluation showed a reduction in ventilation, a decrease in total lung volume, and an increase in venous admixture.

b. (Right) Two months after a pneumonectomy, excision of phrenic nerve and "tailoring" thoracoplasty. Ventilatory function was reduced; respiratory gas exchange improved, as shown by a decrease in venous admixture.

trachea to the right, pleural thickening, pleural effusion, and approximately 70 per cent collapse of the right lung. The left lung appeared clear. Bronchospirometry revealed poor ventilation and no evidence of oxygen uptake on the diseased side. Physiologic evaluation at this time showed reduction in ventilatory function, a decrease in total lung volume, and an increase in venous admixture.

A pneumonectomy, excision of the phrenic nerve, and a "tailoring" thoracoplasty were performed. Function studies were repeated two months after the operation. Although the ventilatory function was reduced, there was a definite improvement of respiratory gas exchange as shown by a decrease in venous admixture.

Pleurisy with effusion:

Case 24. D. V., a 24-year-old white female, with moderately advanced pulmonary tuberculosis developed pleurisy with effusion on the right. Physiologic evaluation three weeks after the onset of the pleurisy revealed a decrease in lung volume measurements and in ventilatory function, as well as an increase in venous admixture.

Peritracheal tuberculous lymphadenitis:

Case 25. H. S., a 24-year-old Negro, was hospitalized as a diagnostic problem. A chest roentgenogram on admission revealed enlarged lymph nodes in the right hilum and right upper mediastinal area. Bronchography and bronchoscopy findings were normal. Physiologic measurements made shortly after admission showed decreased ventilatory function, increased residual volume, arterial oxygen unsaturation, and an increase in venous admixture. Gastric washings cultured at the time of admission were positive for tubercle bacilli.

DISCUSSION

An interplay of the forces of destruction and repair are constantly taking place in pulmonary tuberculosis. The variations of this disease process are endless and, consequently, no specific single type of respiratory impairment is typical. In minimal pulmonary tuberculosis, in which the lesion is confined to a relatively small area, no abnormalities of ventilation and lung volume measurements are demonstrable. However, if the disease process occupies a segment or more, a slight increase in venous admixture may be discernible.

In moderately advanced and far advanced pulmonary tuberculosis, in which the process of destruction is more extensive, impairment of respiratory function is commonly seen.

An increase in the residual volume, RV/TLC ratio of more than 35 per cent, and a reduction in the maximal breathing capacity have been widely used as an expression of pulmonary distention, suggestive of pulmonary emphysema. Using this criterion, there is a greater incidence of an emphysema pattern in patients with far advanced tuberculosis than in those with moderately advanced tuberculosis. In *Cases 1* to *4* of the present study, the high RV/TLC ratio was also associated with a low oxygen diffusion capacity. This is further evidence of the presence of emphysema. It is not possible to determine whether the emphysema existed prior to the onset of the tuberculous process or developed as a result of the disease. A determination of the residual volume on 40 patients with far advanced pulmonary tuberculosis revealed an incidence of 58 per cent with an RV/TLC ratio of more than 35 per cent and only 38 per cent of 26 patients with a similar finding in the group with moderately advanced disease (20). These findings are

thought to indicate that the emphysema is of secondary nature to the tuberculous process in most instances. The presence of emphysema is further substantiated by a greater decrease in the maximal breathing capacity than in the vital capacity.

Case 5 merits special consideration in that this patient had excellent ventilatory function with a marked increase in the residual volume and its ratio. It is conceivable that this represented a simple overdistention of the lung not characterized by obstruction or loss of lung elasticity. Excluding this case, all of the other patients with a RV/TLC of 35 per cent or more showed an increase in dead-space ventilation and in venous admixture, while 4 of 5 revealed an impairment of the oxygen diffusion capacity.

The impairment of the diffusion capacity in these patients is perhaps due to a reduction in the effective alveolar diffusion surface. The increase in venous admixture and in dead-space ventilation is due to an abnormal distribution of air and blood in the lungs.

Case 10 also illustrates another interesting situation. In spite of normal ventilatory function and a normal residual volume, a marked increase in venous admixture and a decreased oxygen diffusion capacity of the alveolar membrane were observed. It should be borne in mind that normal ventilatory function and lung volume measurements do not guarantee normal respiratory gas exchange.

It is well accepted that pulmonary tuberculosis causes an obliterative endarteritis (21). The finding of an increased venous admixture in pulmonary tuberculosis would indicate the presence of alveoli which are poorly ventilated but well perfused, and would also indicate that the endarteritis is incomplete. The extent of the disease process in which an increase in venous admixture could be detected was at least one segment or more. This does not imply, however, that the abnormality is always present in disease occupying an area greater than one pulmonary segment. It would depend to some extent upon the completeness of the obliterative endarteritis. An increase in venous admixture was slight in the presence of a widely scattered fine nodular infiltration such as was seen in patients with far advanced pulmonary tuberculosis (*Cases 7* and *8*).

It is of interest to note that the pleural involvement of one lung, as seen in *Cases 22*, *23*, and *24*, gave approximately the same or a greater degree of impairment in respiratory gas exchange as did miliary tuberculosis.

In the patient with peritracheal lymphadenopathy (*Case 25*) studies revealed an increase in venous admixture and arterial oxygen unsaturation out of proportion to the disease process present. The possible obstruction created by the lymphadenopathy and the emphysema were insufficient to account for this abnormality. A direct intracardiac or intrapulmonary shunt was suspected; nevertheless, inhalation of pure oxygen for fifteen minutes yielded full oxygen saturation of the arterial blood. It is possible that this patient had more pulmonary involvement than was evident by roentgenographic and clinical examinations.

No definite relationship between age, known duration of the disease, and the findings of physiologic testing was observed. In one patient with miliary tuberculosis a marked improvement of diffusion was noted after three months of

chemotherapy. Several other patients studied on two occasions while receiving chemotherapy failed to show any appreciable change in their findings, in spite of roentgenographic evidence of resolution. This suggests that chemotherapy administered for two to three and one-half months does not significantly reverse ventilation-perfusion impairment in that period of time.

Following a pneumonectomy, the patient of *Case 23* showed improvement in arterial oxygen saturation, decrease in venous admixture and dead-space ventilation in the presence of reduced ventilatory function, and increased residual volume.

The improvement in arterial oxygen saturation and decrease in venous admixture and in dead-space ventilation may have resulted from the removal of poorly functioning lung. On the other hand, the reduction in ventilation which is not to be expected by removal of poorly ventilating lung may have been caused by an impairment of rib and diaphragmatic motion resulting from the right thoracoplasty and phrenicectomy. In contrast, in *Case 22*, in which a decortication, resection of residual disease, and a "tailoring" thoracoplasty were performed, no improvement in either ventilation or respiratory gas exchange could be demonstrated two months after operation. The failure of gas exchange to improve after decortication in this case may be related to impaired function in the unresected portion of the left lung.

SUMMARY

Pulmonary function studies in 25 selected cases of pulmonary tuberculosis have been presented. The data suggest that unequal alveolar aeration and perfusion characterized by an increase in venous admixture are commonly encountered respiratory abnormalities in pulmonary tuberculosis.

A close relationship between the extent of the disease process and the development of a pulmonary emphysema pattern, characterized by an increase in the residual volume and a reduction in the maximal breathing capacity, was observed.

Patients with chronic pulmonary tuberculosis studied while receiving chemotherapy failed to reveal any significant change in ventilation-perfusion relationships when these studies were repeated two to four months later.

Respiratory impairment in several special types of tuberculosis involvement was presented and discussed.

SUMARIO

Estudios del Deterioro de la Función Respiratoria en la Tuberculosis Pulmonar

Preséntanse estudios de la función pulmonar en 25 casos seleccionados de tuberculosis pulmonar. Los datos sugieren que una aereación y una perfusión alveolares irregulares caracterizadas por aumento de la mezcla venosa son anomalías respiratorias encontradas comúnmente en la tuberculosis pulmonar.

Observóse una relación íntima entre la extensión del proceso morboso y la aparición de un patrón de enfisema pulmonar, caracterizado por aumento del volumen residual y reducción de la capacidad respiratoria máxima.

Los enfermos que tenían tuberculosis pulmonar crónica, estudiados mientras recibían quimioterapia, no revelaron mayor alteración de las relaciones entre ventilación y perfusión al repetirse estos estudios de dos a cuatro meses después.

Discútese el deterioro respiratorio en varias formas especiales de afección tuberculosa.

RESUME

Recherches sur l'alteration de la fonction respiratoire dans la tuberculose pulmonaire

Présentation d'une étude de la fonction respiratoire dans la tuberculose pulmonaire dans 25 cas sélectionnés. Les données suggèrent que la ventilation et la circulation alvéolaire inégales caractérisées par l'accroissement du mélange de sang veineux sont des anomalies fréquemment rencontrées dans la tuberculose du poumon. Les auteurs ont observé une corrélation étroite entre l'étendue du processus pathologique et le développement des caractéristiques de l'emphysème pulmonaire, ce'est à dire l'augmentation du volume résiduel et la réduction de la capacité respiratoire maxima.

Les cas de tuberculose pulmonaire chronique, étudiés au cours de la chimiothérapie, n'ont pas montré une modification appréciable des rapports ventilation-circulation, quand ces recherches ont été reprises de deux à quatre mois plus tard.

L'altération de la fonction respiratoire dans plusieurs types particuliers de lésions tuberculeuses est décrite et discutée.

Acknowledgments

The writers wish to give grateful acknowledgment to Miss Dimite Buckley, Mrs. Robert Macmillan, and Mr. Samuel Fraley for their technical assistance in certain phases of these studies.

REFERENCES

(1) Riley, R. L., and Cournand, A.: Analysis of factors affecting concentrations of oxygen and carbon dioxide in gas and blood of lungs: Theory, J. Appl. Physiol., 1951, *4*, 77.

(2) Riley, R. L., Donald, K. W., and Cournand, A.: Analysis of factors affecting concentrations of oxygen and carbon dioxide in gas and blood of lungs: Methods, J. Appl. Physiol., 1951, *4*, 102.

(3) Donald, K. W., Renzetti, A., Riley, R. L., and Cournand, A.: Analysis of factors affecting concentrations of oxygen and carbon dioxide in gas and blood of lungs: Results, J. Appl. Physiol., 1952, *4*, 497.

(4) Austrian, R., McClement, J. H., Renzetti, A. D., Jr., Riley, R. L., Donald, K. W., and Cournand, A.: The clinical and physiological features of some types of pulmonary disease with impairment of alveolar-capillary diffusion: The syndrome of "alveolar-capillary block", Am. J. Med., 1951, *11*, 667.

(5) McClement, J. H., Renzetti, A. D., Himmelstein, A., and Cournand, A.: Cardiopulmonary function in the pulmonary forms of Boeck's sarcoid and its modification by cortisone therapy, Am. Rev. Tubere., 1953, *67*, 154.

(6) Stone, D. J., Schwartz, A., Feltman, J. A., and Lovelock, F. J.: Pulmonary function in sarcoidosis, Am. J. Med., 1953, *15*, 468.

(7) Williams, M. H., Jr.: Pulmonary function in Boeck's sarcoid, J. Clin. Investigation, 1953, *32*, 909.

(8) Carroll, D., Cohn, J. E., and Riley, R. L.: Pulmonary function in mitral valvular disease: Distribution and diffusion characteristics in resting patients, J. Clin. Investigation, 1953, *32*, 510.

(9) Williams, M. H., Jr.: Pulmonary function studies in mitral stenosis before and after commissurotomy, J. Clin. Investigation, 1953, *32*, 109.

(10) McClement, J. H., Renzetti, A. D., Jr., Carroll, D., Himmelstein, A., and Cournand, A.: Cardiopulmonary function in hematogenous pulmonary tuberculosis in patients receiving streptomycin therapy, Am. Rev. Tubere., 1951, *64*, 583.

(11) Anderson, L. L., Bell, J. C., and Blount, S. G., Jr: An evaluation of factors affecting the alveolar-arterial oxygen tension gradient in chronic pulmonary disease, Am. Rev. Tubere., 1954, *69*, 71.

(12) Baldwin, E. deF., Cournand, A., and Richards, D. W., Jr.: Pulmonary insufficiency: I. Physiological classification, clinical methods of analysis, standard values in normal subjects, Medicine, 1948, *27*, 243.

(13) Motley, H. L.: Clinical pulmonary physiology: II. Detection of early lung function changes in industrial exposure, Indust. M. Surg., 1953, *22*, 262.

(14) Darling, R. C., Cournand, A., and Richards, D. W., Jr.: Studies on intrapulmonary mixture of gases: III. Open circuit method for measuring residual air, J. Clin. Investigation, 1940, *19*, 609.

(15) Kaltreider, N. L., Fray, W. W., and Hyde, H. W.: Effect of age on pulmonary capacity and its subdivisions, Am. Rev. Tuberc., 1938, *37*, 662.

(16) Riley, R. L., Proemmel, D. D., and Franke, R. E.: A direct method for determination of oxygen and carbon dioxide tensions in blood, J. Biol. Chem., 1945, *161*, 621.

(17) Van Slyke, D. D., and Neill, J. M.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurement, J. Biol. Chem., 1924, *61*, 523.

(18) Van Slyke, D. D., and Sendroy, J., Jr.: Studies of gas and electrolyte equilibria in blood: XV. Line charts for graphic calculations by the Henderson-Hesselbalch equation, and for calculating plasma carbon dioxide content from whole blood content, J. Biol. Chem., 1928, *79*, 781.

(19) Pappenheimer, J. R.: Standardization of definitions and symbols in respiratory physiology, Federation Proc., 1950, *9*, 602.

(20) Tomashefski, J. F., and Anno, H.: Unpublished data.

(21) Birkelo, C. C. and Brosius, W. L.: Roentgen visualization of pulmonary arterial circulation in autopsy material, Radiology, 1938, *31*, 261.

PULMONARY RESECTION FOR TUBERCULOSIS[1,2]

Experience with 200 Consecutive Patients

KENNETH CHIACHE SZE,[3] A. SAMADI, AND JAMES S. CONANT

(Received for publication August 31, 1954)

INTRODUCTION

In recent years, we have witnessed an era of rapidly changing indications for pulmonary resection for tuberculosis. Since the advent of antituberculous chemotherapy, resection is emerging as the surgical treatment of choice in tuberculosis, replacing collapse therapy (1, 2). Surgical excision, when applicable, has become an accepted part of an integrated program of treatment in pulmonary tuberculosis along with long-term chemotherapy and rehabilitation.

This paper presents a clinical and statistical analysis of 201 consecutive pulmonary resections in 200 patients at the Glenn Dale Hospital between July, 1949, and March, 1954. The indications for pulmonary resection, the type of operation, postoperative complications, and the results will be discussed.

GENERAL BACKGROUND OF OPERATIVE GROUP

Age, Sex, and Race

In the 200 cases, there were 66 white patients, of whom 21 were male and 45 female; 130 Negroes, of whom 47 were male and 83 female; and 4 male Orientals were included. The age, sex, and race distribution is shown in figure 1. Females predominated in the early age groups, but, in those more than forty years of age, the distribution between the sexes was approximately equal.

Procedures Employed

All patients undergoing pulmonary resection for tuberculosis were treated with one or more antimicrobial drugs in various combinations for various periods. The drugs used were streptomycin, isoniazid, and para-aminosalicylic acid (PAS). Early in the series, it was believed that one to three weeks of such treatment preoperatively was sufficient. In the more recent cases, resection was withheld until chemotherapy had produced maximum resolution of the lesion as demonstrated by roentgenogram. Chemotherapy was continued for at least six months after the operation.

Individual ligation of the hilar structures was employed in 200 instances. In one right lower lobectomy, a mass ligature technique was necessitated by densely scarred hilus. All operations except three were performed in the lateral recumbent position. The exceptions were done in the prone position.

[1] From the Surgical Service, Glenn Dale Hospital, Glenn Dale, Maryland; and Department of Public Health, District of Columbia.

[2] Presented at the meeting of the Eastern Section of the American Trudeau Society, Washington, D. C., October, 1954.

[3] Present address: 34th General Hospital, APO #58, % Postmaster, New York, New York.

All resections, except seven, were performed in one stage. These seven two-stage cases consisted of two pneumonectomies, four upper lobectomies, and one segmental resection. These cases were staged either because difficult dissection required an unusually long time or because the patient's general condition would not permit the surgeon to complete the operation safely.

One patient had a concomitant left upper lobectomy and thoracoplasty because of a destroyed lobe. Five months later, in the superior segment of the left lower lobe, a cavity appeared and a superior segmental resection was subsequently performed.

The types of pulmonary resection are listed in table 1. "Upper lobectomy" includes upper lobe resection alone or with concomitant or subsequent thoracoplasty, superior segmental resection, or a wedge resection of another lobe.

Indications for Resection

The indications for pulmonary resection for tuberculosis which were used are classified into seven groups, as listed in table 2.

Persistent cavitary disease: In the category of persistent cavitary disease were the cases in which cavitation persisted in spite of roentgenographic improvement or stability. Those patients with giant or tension cavities were included. This was the most common indication for resection of one or part of one lung or lobe. In the 45 earlier cases of this series, pulmonary resection was preceded by unsuccessful collapse therapy, such as pneumothorax, pneumoperitoneum, or phrenic crush. However, thoracoplasty failures were excluded from this group.

Destroyed lung or lobe: The destroyed lung or lobe group included those cases in which one lung or lobe was extensively involved, usually with multiple cavities, bronchiectasis, and atelectasis. Many of the cases were the end result of medically treated extensive bilateral disease. Surgery was indicated for the purpose of removing that lobe which continued to be the major source of infection.

Thoracoplasty failure: Those patients who had persistent cavitary disease with sputum positive for tubercle bacilli, in spite of a satisfactory anatomic collapse by thoracoplasty, fell into the group. In none of the cases was failure of thoracoplasty due to inadequate surgery, such as failure to remove transverse processes, insufficient rib resection, or long anterior stumps. It is believed that the thoracoplasties failed because of giant cavities, spread of disease to the base, endobronchial disease, or bronchiectasis (3).

Residual caseo-necrotic disease: The residual caseo-necrotic focus or foci which remained in the lung after maximum resolution following chemotherapy constituted another indication for pulmonary resection. Tuberculomas were included in this group. The decision for pulmonary resection in this group of patients was most difficult to make. When the lesion had reached the "therapeutic target point" (4), the debatable question was always surgical extirpation of the disease versus continued chemotherapy. If the patient fell into one or more of the following categories, history of reactivation of pre-existing disease with or without prior chemotherapy, poor socio-economic condition, or failure of the patient to cooperate in medical treatment, pulmonary resection was recommended.

TABLE 1

Type of Operations and Postoperative Complications

	Number of Operations	Atelectasis	Broncho-pleural Fistula	Empyema	Shock	Spread	Reactivation	Pneumothorax	Hemothorax	Pulmonary Edema	Pleural Effusion	Massive Pulmonary Embolus	Tuberculous Sinus	Temporary Phrenic Paralysis	Cor Pulmonale	Pericardial Effusion	Pneumonia, Nontuberculous	Postoperative Psychosis	Radial Nerve Palsy	Thrombophlebitis	Operative Deaths
Right pneumonectomy	16		2	2	2					1			2			1					2
Left pneumonectomy	23		1	1		4	1			2		1									2
Total	39		3	3	2	4	1			3		1	2			1					4
Percentage			7.7	7.7	5.1	10.3	2.6			7.7		2.6	5.1			2.6					10.3
Right upper lobectomy	72	9	6	2	2			1	2	1	1			1			1	1			1
Right upper and right middle lobectomy	8	2	1	1			1							1	1						1
Right middle lobectomy	1																				
Right lower lobectomy	3										1										
Left upper lobectomy	41	5	2	1	2		1	1	1										1	1	
Left lower lobectomy	7	1					1				1										
Total	132	17	9	4	4		3	2	3	1	3			2	1		1	1	1	1	2
Percentage		12.9	6.8	3	3		2.3	1.5	2.3	0.8	2.3			1.5	0.8		0.8	0.8	0.8	0.8	1.5
Segmental and wedge resection	30	1	4	1				2	1			1									1
Percentage		3.3	13.3	3.3				6.7	3.3			3.3									3.3
Grand Total	201	18	16	8	6	4	4	4	4	3	3	2	2	2	1	1	1	1	1	1	7
Percentage	100	9	8	4	3	2	2	2	2	1.5	1.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	3.5

TABLE 2

INDICATIONS FOR PULMONARY RESECTION

Persistent cavitary disease	129
Destroyed lung or lobe	24
Thoracoplasty failure	16
Residual caseo-necrotic disease	15
Bronchostenosis	8
Diagnostic problems	7
Bronchiectasis	2
Total	201

Bronchostenosis: The group with bronchostenoses included patients with active pulmonary tuberculosis complicated by considerable narrowing of the main or a lobar bronchus as demonstrated by bronchoscopy.

Diagnostic problems: In this group of 7 cases, the primary indication for thoracotomy was suspected malignant disease of the lung. However, chemotherapy was started prior to surgery and pulmonary resection was performed. Tuberculosis was diagnosed by frozen section and confirmed by paraffin section in all of the 7 cases.

Bronchiectasis: The group with bronchiectasis consisted of 2 patients in whom parenchymal pulmonary tuberculosis was associated with bronchiectasis and persistent hemoptysis.

OBSERVATIONS

Complications

The postoperative complications, as related to the type of pulmonary resections, are listed in table 1.

Atelectasis: Postoperative atelectasis occurred in a total of 18 patients. In each case, the collapse was on the operative side (eleven on the right and seven on the left).

There were eleven concomitant upper lobectomies and thoracoplasties performed on each side. These resulted in postoperative atelectasis in 5 patients after left resection, and in 2 patients after right resection.

All patients with atelectasis were treated with bronchoscopic aspiration, resulting in complete re-aeration of the lung.

Bronchopleural fistula and empyema: Sixteen patients developed bronchopleural fistulas following pulmonary resection. These are classified into three categories:

Eight patients had bronchopleural fistulas and empyemas. Six were successfully treated by open drainage of the empyema and thoracoplasty. Aspiration combined with thoracoplasty controlled this condition in another patient. The remaining patient had a sufficient collapse from an earlier thoracoplasty to permit spontaneous healing of the post-pneumonectomy bronchopleural fistula.

In 5 patients, persistent leakage of air without frank empyema required water-sealed drainage for periods of seven to fourteen days. In 4 of these, a three- or four-

rib thoracoplasty was ultimately performed to obliterate the potentially infected space. In the fifth, complete re-expansion occurred spontaneously.

Three patients had small fluid pockets remaining in the apices. In each case, the fluid was apparently coughed up spontaneously through a small bronchopleural fistula approximately eight weeks after resection, leaving an apical pneumothorax. The space was closed by thoracoplasty.

Shock: Hemorrhagic shock occurred in 6 patients, either during or within a few hours after the completion of surgery. Two patients failed to respond to transfusions of whole blood and died, following pneumonectomies (deaths No. 1 and No. 2, table 3). Three other patients responded readily to transfusions of compatible whole blood. One patient, with adequate blood replacement, developed profound shock three hours after a concomitant right upper lobectomy and thoracoplasty and failed to respond to further blood transfusions. Intravenous nor-epinephrine and intramuscular cortisone resulted in complete recovery from the shocklike state.

Spread and reactivation of tuberculosis: Spread indicated the appearance, during the immediate postoperative period, of new infiltration, presumably tuberculosis, in roentgenograms of the chest. All four "spreads" occurred following left pneumonectomies. One patient died (death No. 4, table 3) and, in the other three, the disease became inactive after long-term chemotherapy.

Four patients had reactivation of the pre-existing disease: 2 in the ipsilateral side and the other 2 in the contralateral side. Three recovered, and one died of cor pulmonale (death No. 7, table 3).

Spontaneous pneumothorax: In 4 patients, the lung re-expanded completely within twenty-four to forty-eight hours following the resection, and the subsequent roentgenograms of the chest showed complete re-expansion of the lung prior to the development of spontaneous pneumothorax. In each case, the patient suddenly was seized with sharp pain in the chest eight or more days after the operation. The chest films demonstrated a pneumothorax, distant from the site of pulmonary resection, where the lung appeared to be completely expanded and adherent. Two patients required no treatment. The lungs in the other 2 patients re-expanded completely with closed drainage for two or three days.

Hemothorax: Hemothorax occurred in 4 patients. A considerable amount of blood drained through the intercostal tubes, and an apical pocket of hemothorax remained. In one patient, no further therapy was needed. In 2 other patients, bronchopleural fistulas developed, closed spontaneously, and four-rib thoracoplasties were performed with good results. In the fourth patient, trypsin was injected into the hemothorax on the twentieth postoperative day. A bronchopleural fistula and empyema developed within twelve hours and were successfully treated by open drainage and a seven-rib thoracoplasty.

Pulmonary edema: Four patients developed pulmonary edema within twelve hours after resection. Three patients died in spite of vigorous treatment with rapid digitalization, morphine sulfate, oxygen with or without positive pressure, and bloodless phlebotomy. In the fourth patient, although the estimated blood loss was recorded as 1,500 cc., a total of 2,500 cc. of whole blood, 500 cc. of five

TABLE 3

ANALYSIS OF DEATHS

Number	Age, Race and Sex	Type of Operation	Indication for Operation	Time of Death After Operation	Cause of Death	Autopsy
			Postoperative Deaths			
1	40 NF	Right pneumonectomy	Thoracoplasty failure	4 hours	Shock due to hemorrhage	Yes
2	35 NM	Right pneumonectomy	Thoracoplasty failure	5 hours	Shock due to hemorrhage and pulmonary edema	Yes
3	31 WF	Right upper lobectomy	Thoracoplasty failure	20 hours	Pulmonary edema	Yes
4	50 NF	Left pneumonectomy	Destroyed lung	3 days	Pulmonary edema and tuberculous pneumonia of right lung	Yes
5	49 NM	Left superior segmental resection	Persistent cavitary disease	14 days	Massive pulmonary embolus	Yes
6	37 WF	Left pneumonectomy	Destroyed lung	15 days	Massive pulmonary embolus from pelvic veins	Yes
7	41 NM	Right upper and middle lobectomy	Thoracoplasty failure	20 days	Cor pulmonale and tuberculous pneumonia of right lower lobe	Yes
			Late Deaths			
8	45 WM	Right upper lobectomy	Persistent cavitary disease	8 months	Squamous cell carcinoma of left lung, inoperable, brain metastasis	Yes
9	44 WM	Left upper lobectomy	Destroyed lung	10 months	Cause unknown—sudden death 3 days after disciplinary discharge; known alcoholic	No
10	39 WF	Right pneumonectomy	Destroyed lung	14 months	Acute adrenal insufficiency after bilateral adrenalectomy for Cushing's syndrome	No
11	28 NF	Right pneumonectomy	Destroyed lung	37 months	Tuberculous pneumonia of left lung	Yes

per cent dextrose in water, and 400 cc. of normal saline were inadvertently given during the left pneumonectomy. This patient recovered following treatment.

Pleural effusion: An excessive amount of apparently sterile intrapleural fluid developed in 3 patients, requiring repeated thoracenteses. In one patient, the effusion continued to form until the apical hydropneumothorax was closed by a four-rib thoracoplasty. All 3 patients recovered from this complication.

Miscellaneous complications: The 2 patients with massive pulmonary emboli died (deaths Nos. 5 and 6, table 3). One patient developed progressive cor pulmonale and died twenty days postoperatively (death No. 7, table 3).

Two patients developed tuberculous sinus tracts in the incision one and twelve months following left pneumonectomies. One was treated conservatively with antituberculous chemotherapy; and the other, by excision. Both patients recovered.

All patients who developed the other complications listed in table 1 recovered.

Postoperative deaths: In the present series, there were 7 (3.5 per cent) postoperative deaths in 201 consecutive pulmonary resections for tuberculosis (tables 3 and 4). All occurred within sixty days following surgery.

Therapeutic Results

It was not possible to locate 5 of the 200 patients following their discharge from the hospital. As there were 7 postoperative deaths, the subsequent observation is reported on 188 patients as of August, 1954.

The length of follow-up and the information available on these 188 patients are indicated in table 5. The follow-up status as related to the type of resection is given in table 6.

One hundred and fifty-seven patients (83.5 per cent) had no tuberculous complications during the follow-up period. Their serial roentgenograms of the chest, at regular intervals, show normal pulmonary findings or do not show any active tuberculosis. Four patients had late spread or reactivation of pre-existing disease during the follow-up period, and their pulmonary tuberculosis is classified as inactive or arrested.

The work status of 161 patients, 157 with no tuberculous complications and 4 with late spread of disease is as follows: 58 patients are doing full-time work; and 15, part-time work. The work status of the remaining 88 patients is undetermined.

TABLE 4

MORTALITY AS RELATED TO TYPE OF OPERATION

Type of Operation	Number of Operations	Postoperative Deaths	Late Deaths	Total Mortality
Pneumonectomies	39	4 (10.3%)	2 (5.1%)	6 (15.4%)
Lobectomies	132	2 (1.5%)	2 (1.5%)	4 (3.0%)
Segmental and wedge resections	30	1 (3.3%)	0	1 (3.3%)
Totals	201	7 (3.5%)	4 (2.0%)	11 (5.5%)

TABLE 5

FOLLOW-UP STATUS OF 188 PATIENTS

Duration Observed	Number	Uncomplicated	Late Spread of Disease; Patient Now Well	Hospitalized	Active Disease; Not Hospitalized	Deaths
6 to 12 months	67	51 (76.1%)	0	12 (17.9%)	2 (3.0%)	2 (3.0%)
1 to 2 years	70	65 (92.9%)	0	3 (4.3%)	1 (1.4%)	1 (1.4%)
2 to 3 years	30	27 (90.0%)	0	2 (6.7%)	1 (3.3%)	0
3 to 4 years	14	8 (57.2%)	3 (21.5%)	1 (7.1%)	1 (7.1%)	1 (7.1%)
4 to 5 years	6	5 (83.3%)	1 (16.7%)	0	0	0
5 to 6 years	1	1 (100%)	0	0	0	0
Total	188	157 (83.5%)	4 (2.1%)	18 (9.6%)	5 (2.7%)	4 (2.1%)

TABLE 6

FOLLOW-UP STATUS AS RELATED TO TYPE OF RESECTION

	Number of Patients Observed	Uncomplicated	Late Spread of Disease; Patient Now Well	Hospitalized	Active Disease; Not Hospitalized	Late Deaths
Pneumonectomy	33	26 (78.8%)	1 (3.0%)	3 (9.1%)	1 (3.0%)	2 (6.1%)
Lobectomy	126	108 (85.7%)	2 (1.6%)	11 (8.7%)	3 (2.4%)	2 (1.6%)
Segmental and wedge resection	29	23 (79.4%)	1 (3.4%)	4 (13.8%)	1 (3.4%)	—
Total	188	157 (83.5%)	4 (2.1%)	18 (9.6%)	5 (2.7%)	4 (2.1%)

TABLE 7

ANALYSIS OF THE 18 STILL HOSPITALIZED PATIENTS

Length of Follow-up	Number of Patients	I	II	III	IV
6 to 12 months	12	4	6	1	1
1 to 2 years	3	1	2	0	0
2 to 3 years	2	0	0	0	2
3 to 4 years	1	0	0	0	1
Total	18	5	8	1	4

I. Prolonged hospitalization because of pre-existing extensive bilateral tuberculosis.

II. Prolonged hospitalization because of postoperative complications, now completely controlled.

III. Contralateral pulmonary resection for pre-existing tuberculosis.

IV. Late spread or reactivation of pre-existing tuberculosis.

The 18 hospitalized patients are classified into the following four categories (table 7):

I. Five patients have had prolonged hospitalization because of pre-existing extensive bilateral tuberculosis. They are all being rehabilitated and will be discharged in the near future.

II. Eight patients have had prolonged hospitalization because of postoperative complications.

III. One patient required contralateral upper lobectomy for pre-existing tuberculosis.

IV. Four patients are hospitalized for late spread or reactivation of pre-existing tuberculosis.

Five patients still have active pulmonary tuberculosis but are not hospitalized (table 5). Two of the 5 are improving under chemotherapy administered on an ambulatory basis.

Nine Negroes and 2 white patients had late spread or reactivation of pre-existing tuberculosis during the follow-up period. Their present status is as follows: 4 patients are still hospitalized and have shown consistent roentgenographic improvement; one has pulmonary tuberculosis classified as arrested; 3 are classified as inactive; 2, active improved; and one is dead.

There are 4 late deaths (tables 3, 4, and 5), only one of which was due to pulmonary tuberculosis.

DISCUSSION

The age, color, and race of the patients in the present series (figure 1) indicate the differences between tuberculosis in the Negro and white and between the two sexes. These differences can be summarized as follows (5, 6): In the Negro, the younger age groups are relatively more affected; and in the younger groups, the females are more affected; in the older groups, the males.

The young females, Negro and white, made up the largest group of resections in this series. This was due to the great incidence of acute caseo-pneumonic disease successfully controlled with chemotherapy. The remaining cavitation was suitable for resection (7, 8).

In the earlier cases of the present series, a three- or four-rib thoracoplasty was performed routinely following an upper lobectomy in order to prevent over-

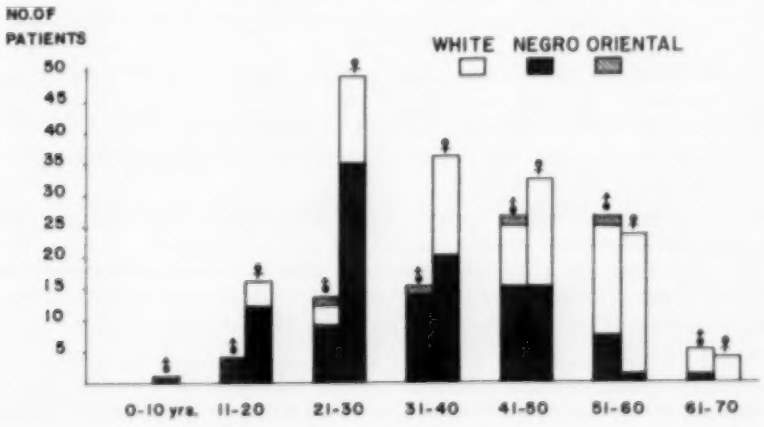


FIG. 1. Age, sex, and race of 200 patients.

distension of the remaining lung and reactivation of the residual disease. At the present time, with the protection of postoperative chemotherapy for at least six months, a prophylactic thoracoplasty is no longer performed as a routine. Instead, the procedure is reserved for the patient with a residual pleural space caused by incomplete re-expansion of the remaining lung, or with a bronchopleural fistula. A five- or six-rib thoracoplasty is still performed following a pneumonectomy, however, in order to avoid late cardiorespiratory symptoms.

In this series all complications which occurred within sixty days after pulmonary resection were included. Most of the papers in recent literature (9–12) only list the principal complications following surgical extirpation; that is, bronchopleural fistula, empyema, spread or reactivation of pre-existing disease, and tuberculous infection of the chest wall.

The incidence of bronchopleural fistula and empyema in the present series is relatively high, especially in lobectomies (6.8 per cent and 3 per cent respectively, table 1). Of 3 bronchopleural fistulas following hemothorax, 2 developed empyema. It is believed that, if subsequent thoracotomies were performed as soon as the patient's general condition permitted in order to evacuate the blood clots and, if necessary, to decorticate the lung, these complications might not have occurred.

It has been generally believed that, when a bronchopleural fistula develops, the infected intrapleural fluid is suddenly released into the bronchial tree to be followed by spread of disease. No "spreads" could be demonstrated in the fistula cases of the present series, nor has the occurrence of bronchopleural fistula to date shown late untoward effect upon the present status of the patients.

Six Negroes and 2 white patients developed reactivation of the pre-existing disease or new "spread" in the postoperative period. Four postoperative "spreads" (table 1) occurred following pneumonectomies performed in lateral recumbent position. It is possible that these "spreads" might not have occurred if the prone position had been employed. One death (death No. 4, table 3) might have been avoided also (13).

SUMMARY

Two hundred and one consecutive pulmonary resections in 200 patients performed between July, 1949, and March, 1954, were studied. In the 200 cases, there were 130 Negroes, 66 white patients, and 4 Orientals. Females predominated in the early age groups, but sex distribution was approximately equal in those more than forty years old.

Antituberculous chemotherapies were employed in all cases in various combinations and for various periods.

The indications for pulmonary resection were persistent cavitary disease, destroyed lung or lobe, thoracoplasty failure, residual caseo-necrotic disease, bronchostenosis, diagnostic problems, and bronchiectasis. Each indication was discussed.

Seven two-stage operations were performed, with good results. The types of pulmonary resection and the complications in relation to each type were listed.

Management of the complications was discussed. The principal tuberculous complications were as follows: bronchopleural fistula, 8 per cent; empyema, 4 per cent; spread of tuberculosis, 2 per cent; reactivation of pre-existing tuberculosis, 2 per cent; tuberculous infection of the chest wall, 1 per cent. There were 7 postoperative deaths (3.5 per cent) and 4 late deaths (2 per cent). The total mortality was 5.5 per cent.

Excluding the 7 postoperative deaths and 5 patients with no follow-up, 188 patients were traced. Of the 188 patients, 161 are well, 18 are hospitalized, 5 have active pulmonary tuberculosis but are not hospitalized, and 4 patients are dead. The work status of 161 well patients was analyzed.

SUMARIO

La Resección Pulmonar por Tuberculosis: Lo Observado en 200 Enfermos Consecutivos

Este estudio comprende 201 resecciones pulmonares consecutivas ejecutadas en 200 enfermos entre julio de 1949 y marzo de 1954. De los 200, 66 eran de raza blanca y 130 de raza negra y 4 de raza Oriental. Las mujeres predominaban en los grupos de edad más tierna, pero la distribución por sexos era aproximadamente igual en las personas de más de cuarenta años.

En todos los casos, se emplearon agentes quimioterapéuticos antituberculosos en varias combinaciones y por más o menos tiempo.

Las indicaciones para la resección pulmonar fueron afección cavitaria persistente, destrucción de un pulmón o lóbulo, fracaso de la toracoplastia, enfermedad caseo-necrótica residual, broncostenosis, problemas de diagnóstico y bronquiectasia. Se discute cada indicación.

Se ejecutaron siete operaciones en dos tiempos, con buenos resultados. Se enumeran las formas de resección pulmonar verificadas y las complicaciones en relación con cada forma. Se discute la asistencia de las complicaciones. Las principales complicaciones tuberculosas fueron: fístula broncopleural, 8 por ciento; empiema, 4 por ciento; difusión, 2 por ciento; reactivación de una tuberculosis preexistente, 2 por ciento; infección tuberculosa de la pared torácica, 1 por ciento. Hubo 7 muertes postoperatorias (3.5 por ciento) y 4 tardías (2 por ciento). La mortalidad total fué de 5.5 por ciento.

Excluyendo las 7 muertes postoperatorias y 5 enfermos perdidos de vista, se dió con 188 enfermos. De éstos, 161 se hallan bien, 18 están hospitalizados, 5 tienen tuberculosis activa, pero no están hospitalizados, y 4 han fallecido. Se analiza la capacidad para el trabajo de los 161 enfermos sanos.

RESUME

Résection pulmonaire dans la tuberculose: Expérience acquise dans 200 cas consécutifs

Deux cent-une résections pulmonaires consécutives, effectuées chez 200 patients entre juillet 1949 et Mars 1954, ont été étudiées. Ces 200 cas comprenaient 66 blancs et 130 nègres et 4 jaunes. Les femmes prédominaient dans les groupes jeunes, mais la répartition des sexes était sensiblement égale chez les sujets au-dessus de quarante ans.

Les agents chimiothérapeutiques antituberculeux furent employés dans chaque cas en combinaisons et périodes variées.

Les indications de la résection pulmonaire étaient les lésions cavitaires persistantes, la destruction d'un poumon ou d'un lobe, l'échec de la thoracoplastie, les lésions caséo-nécrotiques résiduelles, la sténose bronchique, certains problèmes diagnostiques et la bronchectasie. Chacune des indications est étudiée.

Sept opérations en deux temps ont été effectuées avec de bons résultats. Les procédés de résection pulmonaire du type Tye et les complications en relation avec chacun d'eux

sont enregistrés. Le traitement des complications est discuté. Les principales complications d'ordre tuberculeux sont les suivantes: fistule broncho-pleurale, 8%; empyème, 4%; dissémination, 2%; réactivation d'une tuberculose préexistante, 2%, tuberculisation de la paroi thoracique, 1%. Il y a eu 7 décès post-opératoires (3, 5%) et 4 décès tardifs (5, 5%).

A l'exclusion des 7 décès post-opératoires et de 5 cas n'ayant pu être suivis, 188 malades ont été retrouvés. Parmi ces 188 malades, 161 sont bien portants, 18 sont hospitalisés, 5 ont une tuberculose pulmonaire en activité, mais sans hospitalisation, 4 sont morts. Les auteurs analysent la capacité de travail des malades bien portants.

Acknowledgments

The writers wish to express their sincere gratitude to Drs. Moe Weiss, Morton C. Creditor, and Takashi Aoki for their most helpful assistance in preparing this manuscript; and to Dr. Donald R. Sickler and his staff at Tuberculosis Control Division, Department of Public Health, District of Columbia, for their courtesy in supplying the follow-up data.

REFERENCES

(1) Hughes, F. A., Lowry, C. C., and Polk, J. W.: Thoracoplasty and resection for pulmonary tuberculosis, J. Thoracic Surg., 1953, *25*, 454.

(2) O'Brien, W. B., Wilson, N. J., Armada, O., and Vinzberg, W. V.: Total surgical statistics in the treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1953, *68*, 875.

(3) Meissner, W. A., Overholt, R. H., Wilson, N. J., and Walker, J. H.: The resected post-thoracoplasty lung, Am. Rev. Tuberc., 1949, *60*, 406.

(4) D'Esopo, N., Bernstein, S., Decker, A. M., Raleigh, J. W., and Steenken, W., Jr.: Bacteriology of lesions resected during chemotherapy, Tr. Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, Atlanta, Georgia, February, 1953, p. 229.

(5) Pinner, M.: Pulmonary Tuberculosis in the Adult, Charles C Thomas, Springfield, Illinois, 1945.

(6) Rich, A. R.: The Pathogenesis of Tuberculosis, ed. 2, Charles C Thomas, Springfield, Illinois, 1951.

(7) Mitchell, R. S.: Discussion on background factors in consolidated tables on pulmonary tuberculosis, Tr. Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, St. Louis, Missouri, February, 1954, p. 33.

(8) Amberson, J. B.: A summary discussion of the day's papers, Tr. Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, St. Louis, Missouri, February, 1954, p. 205.

(9) Murphy, J. D., and Becker, B. B.: Intermediary report of 102 streptomycin-protected pulmonary resections, Am. Rev. Tuberc., 1953, *22*, 67.

(10) Kergin, F. G., and Coulthard, H. S.: The surgical treatment of pulmonary tuberculosis, S. Clin. North America, 1954, *34*, 931.

(11) Steele, J. D.: Discussion on the data on surgery in the consolidated tables, Tr. Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, St. Louis, Missouri, February, 1954, p. 269.

(12) Overholt, R. H., Wilson, N. J., and Gehrig, L. J.: The place of pulmonary resection in the treatment of tuberculosis, Dis. of Chest, 1952, *21*, 32.

(13) Overholt, R. H., and Langer, L.: The Technique of Pulmonary Resection, Charles C Thomas, Springfield, Illinois, 1949.

A MICROCOLONIAL TEST FOR THE RECOGNITION OF VIRULENT MYCOBACTERIA[1]

IRVING KRASNOW, LAWRENCE G. WAYNE, AND DAVID SALKIN

(Received for publication August 9, 1954)

INTRODUCTION

Acid-fast bacilli are occasionally cultured from man which, by reason of unusual colonial morphology, rapid growth, or resistance to antimicrobials, may be questioned as to their virulence or etiologic significance. Animal inoculation has been the standard procedure for the determination of the virulence of such organisms. In recent years, several *in vitro* tests have been described which appear to aid in the recognition of virulent mycobacteria. For example, Middlebrook, Dubos, and Pierce (1) observed that the ability to form cords is associated with virulence. Dubos and Middlebrook (2) reported that virulent mycobacteria give rise to a characteristic "cytochemical reaction" with alkaline neutral red solution. Paraf and associates (3) obtained similar results with Nile blue. Bloch (4) noted a time differential in the decolorization of methylene blue by virulent and avirulent mycobacteria, and Wilson, Kalish, and Fish (5) employed four indophenol dyes with similar results. Two other suggested tests include the determination of urease activity (6) and aryl sulphatase activity (7).

Of the phenomena listed above, the neutral red reaction and cord formation have been the most widely accepted as differentially significant (8–11). Although these two criteria appear to be more reliable than the others, they do not, individually, conform completely with results obtained by animal inoculation. For example, Richmond and Cummings (8) and Morse, Dail, and Olitzky (11) reported organisms which were nonpathogenic for animals but were neutral red positive. On the other hand, Hart and Rees (12) and Richmond and Cummings (8) have studied avirulent strains of tubercle bacilli which were neutral red negative but showed cording. Corded avirulent mycobacteria have also been observed in the present laboratory.

A review of the literature thus shows that an organism may be positive by just one of these criteria and still be nonpathogenic for laboratory animals. On the other hand, the present writers have not encountered any evidence of virulent mammalian tubercle bacilli which were negative by either criterion. There has been only one organism described, the "radish saprophyte" (8), which was avirulent, yet *fully* positive to both tests, and this will be discussed later.

Wayne (13, 15) has recently described a technique for growing tubercle bacilli on molecular filter membranes and staining them with neutral red and alkali. Microscopic examination of such preparations permitted simultaneous observation of both the neutral red cytochemical reaction and of cording, and he sug-

[1] From the Bacteriological Research Laboratory, Veterans Administration Hospital, San Fernando, California; and the Departments of Infectious Diseases and Medicine, School of Medicine, University of California, Los Angeles, California.

gested that this technique might be used as a virulence test. Independently, Krasnow applied the neutral red stain technique to cultures of mycobacteria which were grown on filter paper according to the method of Hoyt, Smith, and Gribkoff (14).

The purpose of the present article is twofold: to describe the application of the neutral red stain technique to intact colonies of mycobacteria grown on the above two matrices, permitting simultaneous observation of the neutral red reaction and of cording; and to evaluate these two phenomena as a combined test for virulence of tubercle bacilli.

MATERIALS AND METHODS

A total of 94 strains of mycobacteria were studied. Dr. Seymour Froman of Olive View Sanatorium supplied 42 of the strains; 11 of these were chromogens received as isolates from "apparently nontuberculous lesions," and the other 31 were well-characterized stock strains of mycobacteria (*See* tables 1 and 2). The identity of none were known to the writers at the time of the tests. The 22 mammalian strains of tubercle bacilli consisted of subcultures of primary isolates from resected pulmonary lesions of tuberculous patients at the San Fernando Veterans Administration Hospital. All but 2 of these patients had received chemotherapy. Thirteen of the freshly isolated strains studied were susceptible to streptomycin, isoniazid, and para-aminosalicylic acid (PAS), 2 were resistant to 100 γ of streptomycin per ml., 5 were resistant to 10 γ of streptomycin, one was resistant to 10 γ of PAS, and one was resistant to 100 γ of streptomycin and 5 γ of isoniazid.

Two types of inocula were used. One was a Dubos broth[2] culture of the organism; the other was a homogenate of colonies grown on Löwenstein-Jensen egg medium and suspended in a 0.5 per cent solution of bovine albumin fraction V[3].

The organisms were grown on one or both matrices, and the staining procedure was modified to suit each of them. The following stain reagents were employed:

NR-6: a saturated solution of neutral red in 95 per cent ethanol, filtered, and acidified with 1 per cent (v/v) of concentrated hydrochloric acid

Na_2CO_3 developer: a 1:10 dilution of saturated aqueous sodium carbonate

NaOH developer: N/10 aqueous sodium hydroxide

It should be noted that, although the sodium hydroxide developer has been used for the filter paper cultures and the sodium carbonate developer for the molecular filter membrane cultures in the experiments described here, the two developers are actually interchangeable, and other alkaline solutions could probably be substituted.

Molecular Filter Method (15)

In this set of experiments, the growth matrix consisted of molecular filter membranes on Löwenstein-Jensen medium in wide-mouth screw-capped jars. The cultures were incubated in an atmosphere saturated with water vapor.

When sufficient growth occurred to be observable under a dissecting microscope, a segment of the matrix was excised and placed, culture side up, on paper pads saturated with the reagents indicated in the sequence described below:

1. NR-6: ten minutes
2. Na_2CO_3 developer: five minutes
3. 95 per cent ethanol: one minute

The segment was then dried at 37°C. for fifteen minutes and placed on a slide with Permount[4] mounting fluid under a coverslip. The mounting fluid rendered the specimen transparent and suitable for microscopic examination at any desired magnification.

---

[2] Prepared from Difco-Dubos Broth Base.

[3] Obtained from Armour Laboratories, Chicago, Illinois.

[4] Obtained from Fisher Scientific Company, New York, New York.

Filter Paper Method

In this set of experiments, the growth matrix consisted of Whatman No. 1 filter paper strips (10 mm. by 45 mm.) on Löwenstein-Jensen medium in 15 ml., screw-capped prescription bottles. The inoculated bottles were incubated at 37°C. The strips were removed from

TABLE 1

RESULTS OF THE MICROCOLONIAL VIRULENCE TEST ON STOCK CULTURES OF MYCOBACTERIA

Organism	"OV" Number*	Microcolonial Test	
		Neutral Red	Cord
Mammalian tubercle bacilli			
M. tuberculosis var. *hominis*			
H37Rv	3,434	+	+
H37Rv	2,808	+	+
H98	3,448	+	+
B88	5	+	+
Ha.	3,463	+	+
Pr.	3,467	+	+
M. tuberculosis var. *bovis*			
Corper	3,450	+	+
Ravenel	2,806	+	+
Trudeau 4228-2	3,998	+	+
Trudeau 4228-3	3,999	+	+
Avirulent variants			
M. tuberculosis var. *hominis*			
H37Ra	3,462	−	−
H37Ra	4,078	+	−
JHB16 Ra	3,437	−	−
Ta.	2,161	−	−
La.	3,479	+	−
BCG			
Paris strain #805, series 2	3,427	+	+
Aronson #26	3,429	+	+
Sur.	3,441	+	+
Rosenthal	3,442	+	+
Saprophytes			
M. smegmatis	3,506	−	−
M. phlei	3,511	−	+
M. phlei 39	3,497	−	−
M. spp. "radish bacillus"†	4,089	+	−
M. spp. 607	3,444	−	−
M. spp. 66	3,451	−	−
Miscellaneous			
M. avium	3,446	−	−
M. avium	3,461	+	−
M. avium	3,465	+	−
M. ulcerans NR	3,473	+	−
M. spp. *M.B.*	3,452	+	±

* OV numbers are Olive View accession numbers.

† Communicable Disease Center, Atlanta, Ga.

TABLE 2

RESULTS OF THE MICROCOLONIAL TEST ON WILD STRAINS OF MYCOBACTERIA, FILTER PAPER METHOD

	Total	Neutral Red Positive Corded	Neutral Red Positive Non-Corded	Neutral Red Negative Corded	Neutral Red Negative Non-Corded
Mammalian tubercle bacilli from resected lung tissue	22	22	0	0	0
Chromogens from apparently nontuberculous lesions	11	0	10	0	1

the medium in four to eight days and placed on glass slides, which were heat fixed and stained in the following manner:

1. 95 per cent ethanol: one minute
2. NR-6: ten minutes (a few drops of water added to the slide to prevent drying)
3. Stained strip removed from slide, placed on blotting paper, and washed with water, added dropwise
4. NaOH developer: ten drops added and allowed to stand for three minutes
5. Water wash followed by 95 per cent ethanol wash, allowing excess liquid to drain through into blotting paper
6. Dried strip fixed to the slide with plastic cement[5] at each end

The stained strip was rendered transparent and suitable for microscopic examination at any magnification by the application of several drops of immersion oil.

RESULTS

A parallel study of more than 50 *Mycobacterium* cultures using both the molecular filter and filter paper methods showed identical diagnostic results. The molecular filter colonies showed a lesser tendency toward forming "wandering" cords than did those on the paper, but formed more regular and discrete units (*See* figures 1*a* and 2*a*). An advantage of the molecular filter is the feasibility of excising small segments for examination without disrupting the main body of the culture; whereas, in the paper method, the entire strip must be removed as excision of pieces is difficult. The filter paper method showed the advantages of greater economy, greater convenience, and minimal opportunity for contamination. The results tabulated in the present report were obtained by the use of one or both methods.

Based upon the neutral red reaction and the presence of cording, all of the mycobacteria tested were divided into the four possible groups: neutral red positive and corded; neutral red positive but non-corded; neutral red negative but corded; and neutral red negative and non-corded (tables 1 and 2).

Neutral red positive and corded: All of the virulent stock strains of *M. tuberculosis* were neutral red positive and corded. Both properties were also present in the 22 subcultures of tubercle bacilli recovered from resected tissues. The strain of *M. tuberculosis* resistant to 10 γ of PAS per ml. exhibited very loose cording.

[5] General Purpose Cement, Fry Company, Los Angeles, California.

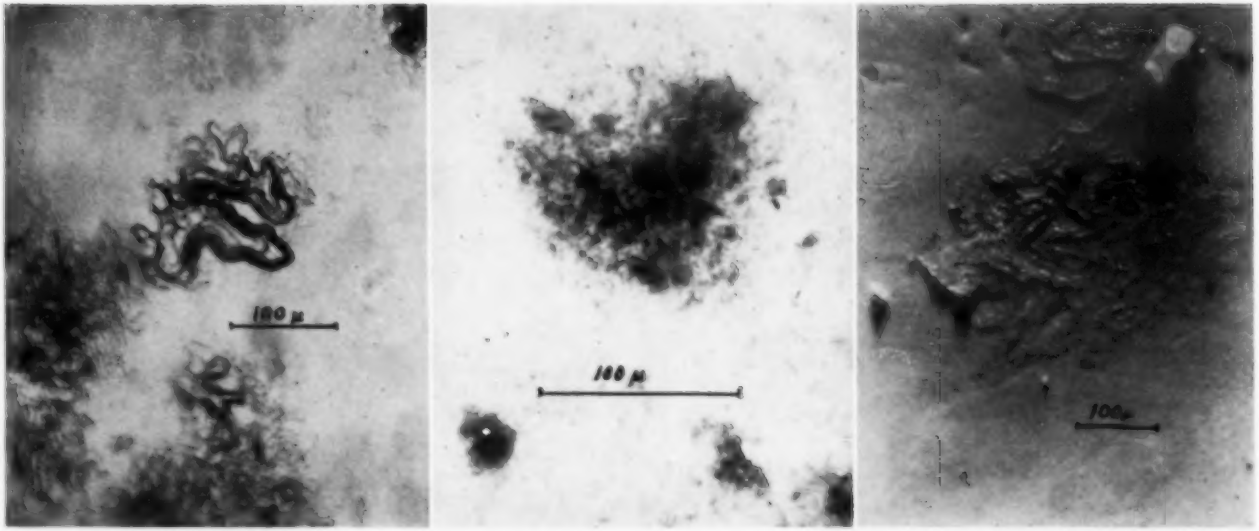


Fig. 1a. (Left) Wild strain of *M. tuberculosis* var. *hominis*, neutral red positive and corded, ten-day-old culture on molecular filter membrane.

Fig. 1b. (Center) Radish saprophyte, neutral red positive but non-corded, four-day-old culture on molecular filter membrane.

Fig. 1c. (Right) *Mycobacterium phlei*, neutral red negative but corded, one-day-old culture on molecular filter membrane, reflected light.

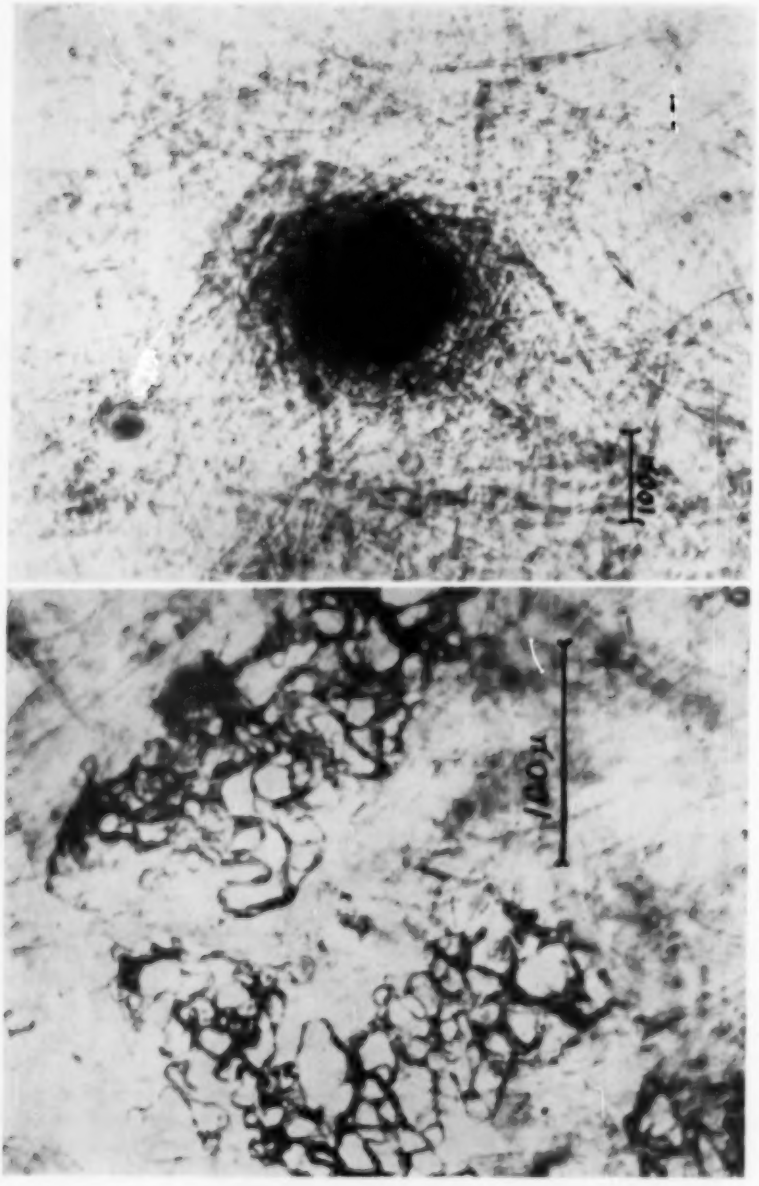


FIG. 2a. (Left) H37Rv, neutral red positive and corded, seven-day-old culture on filter paper strip.
FIG. 2b. (Right) H37Ra, neutral red positive but non-corded, seven-day-old culture on filter paper strip.

Four strains of BCG also fell into this group. Although the BCG cultures were positive by both criteria, they did show considerable variation in the intensity of cording, which was less pronounced than that exhibited by the fully virulent strains of mammalian tubercle bacilli. One organism of questionable pathogenicity, *Mycobacterium* spp. (*M. B.*), also fell into this group.

Neutral red positive but non-corded: The behavior pattern of a positive neutral red reaction in the absence of cord formation was shown by 2 avirulent variants of *M. tuberculosis* var. *hominis*, 2 strains of avian tubercle bacilli, *M. ulcerans*, the radish saprophyte, and 10 of 11 strains of chromogens.

Neutral red negative but corded: M. phlei was the only organism which exhibited cord formation and yet was neutral red negative.

Neutral red negative and non-corded: Neither cord formation nor a positive neutral red reaction was shown by one chromogen, 3 of 5 avirulent variants of *M. tuberculosis* var. *hominis*, 4 saprophytes, and one strain of avian tubercle bacilli.

Figures 1*a*, 1*b*, and 1*c* present typical pictures of the neutral red reaction and cord formation as seen in the different groups on the molecular filter membrane. Figures 2*a* and 2*b* present two of the reactions seen on filter paper cultures.

DISCUSSION

The standard neutral red test of Middlebrook and Dubos gives rise to some variable reactions. In this laboratory, some cultures which have been recorded as positive after one hour in the neutral red buffer suspension have been seen to fade when allowed to stand overnight in buffer without neutral red. A number of other cultures give only partially positive reactions, i.e., some of the areas within a clump of organisms will be red while the remainder will be yellow or white. Chromogens are not amenable to the standard neutral red test because their own color makes the test difficult to read (18).

By means of the technique used in the present study, it has been possible to observe the neutral red reaction at the level of individual bacilli. The organisms which faded in the standard method were neutral red negative when alkalinized by the present method. Some colonies exhibited red color in the center but were yellow at the periphery, and all of the colonies which exhibited this characteristic were non-corded.

The combined test proposed in this paper has the advantage of two virulence tests performed as a single procedure. It permits the simultaneous observation of neutral red reaction and cording on one medium, *in situ*, with no disruption of colonial morphology.

The apparent discrepancy between the findings of Richmond and Cummings (8) and those reported here with respect to the ability of the radish saprophyte to form cords is probably a function of interpretation of terms. Richmond and Cummings apparently decided to classify this organism as corded on the basis of "ropes" of bacilli over the slide ("serpentine" growth), even in the absence of parallel orientation of the cells. On the other hand, the present writers have relied on high-power observation of parallel orientation as the final criterion of

cording. It must be noted that Richmond and Cummings stated that correlation existed between parallel orientation and virulence, but not between "serpentine" growth and virulence.

Although the 4 BCG strains listed in table 1 exhibit the "virulent" reaction, other incomplete data indicate that there are some strains which are either neutral red negative or non-corded. There is considerable variation in the intensity of cord formation exhibited by the BCG strains. Nevertheless, it is not possible to assign quantitative values to this characteristic, as the apparent tightness is partly a function of the physiologic age of a particular colony in a culture. It is not surprising to find this variation in cording, since this phenomenon is so closely associated with virulence. Dubos, Pierce, and Schaefer (16) have recently demonstrated that substrains of BCG differ in their virulence for mice. This observation has long been suggested by the differences one may observe in the response of human hosts to vaccination with BCG cultures from different sources.

Some results of tentative studies on isoniazid-resistant tubercle bacilli indicate that the particular strains involved may be similar to BCG. Organisms resistant to 100 γ of isoniazid per ml. are neutral red positive, exhibit variable cording ability, but fail to produce progressive disease in guinea pigs.

Mycobacterium spp. (*M. B.*), as reported in this paper, was neutral red positive and corded, though avirulent. Here again the intensity of cording was weak, with minimal parallel orientation to be seen.

It is believed that the microcolonial test presented here, is, in its present state, useful diagnostically as a means of interpreting the history of an organism. It is believed that a *Mycobacterium* cultured from a patient which is neutral red positive and corded is derived from a virulent mammalian tubercle bacillus. Even if such an organism is presently incapable of producing progressive disease in animals, the possibility must be considered that it is possessed of a virulent potential. Vorwald, Dworski, and Pratt (17) report the production of progressive disease in silicotic guinea pigs by BCG, but not by H37Ra. They concluded that the former organism is attenuated, while the latter organism is avirulent.

The present writers believe, therefore, that a strain of *Mycobacterium* negative by one or both criteria (neutral red reaction and cord formation) is not a virulent mammalian tubercle bacillus. A strain similarly isolated from clinical material which is neutral red positive and corded may be classified as virulent or potentially virulent.

SUMMARY

An improved procedure has been described for the identification of virulent mycobacteria. It consists of cultivating the organisms on filter paper or molecular filter membrane matrices, treating the microcolonies *in situ* with neutral red and with alkali, and observing them under the microscope. All stock virulent mammalian tubercle bacilli and all strains of tubercle bacilli recovered from resected pulmonary tissues were positive by both criteria. On the other hand, the known saprophytes as well as the chromogens and other mycobacteria of ques-

tionable pathogenicity were either neutral red negative, non-corded, or negative in both respects.

This technique makes it possible to determine simultaneously the cytochemical reaction and the cord-forming ability of microcolonies of mycobacteria.

A brief discussion of the possible clinical significance of the "virulent" reaction in this test is included.

SUMARIO

Prueba Microcolonial para el Reconocimiento de las Micobacterias Virulentas

Descríbese un procedimiento perfeccionado para la identificación de las micobacterias virulentas. Consiste en cultivar los microbios en papel-filtro o matrices de membrana-filtro moleculares, tratando las microcolonias *in situ* con rojo neutro y con álcali y observándolas al microscopio. Todos los bacilos tuberculosos de mamífero virulentos *stock* y todas las cepas extrañas obtenidas de tejidos pulmonares resecados resultaron positivas con ambas normas. En cambio, los saprófitos conocidos así como los cromógenos y otras micobacterias de patogenicidad dudosa resultaron ya negativos al rojo neutro, o sin cordones o negativos en ambos sentidos.

Esta técnica permite determinar simultáneamente la reacción citoquímica y la capacidad para formar cordones de las microcolonias de microbacterias.

Agrégase una breve reseña de la posible importancia clínica de la reacción "virulenta" en esta prueba.

RESUME

Epreuve de la microcolonie (microcolonial test) pour l'identification des mycobactéries virulentes

Description d'une nouvelle technique pour l'identification des mycobactéries virulentes. Elle consiste à cultiver les mycobactéries sur du papier filtre ou sur des membranes filtrantes, à traiter les microcolonies *in situ* avec du rouge neutre et avec de l'alcali et à les observer au microscope. Tous les bacilles tuberculeux bovins de stock et toutes les souches naturelles récupérées dans les tissus pulmonaires réséqués ont donné des résultats positifs avec l'un et l'autre des tests. D'autre part, les saprophytes connus, ainsi que les chromogènes et autres mycobactéries de pathogénicité discutable donnaient une réaction négative au rouge neutre ou ne formaient pas de cordons, ou les deux réactions étaient négatives.

Cette technique permet de déterminer simultanément la réaction cytochimique et l'aptitude des microcolonies bactériennes à former des cordons.

Une brève discussion sur la signification clinique possible de la réaction de virulence dans cette épeuve est incluse.

REFERENCES

(1) Middlebrook, G., Dubos, R. J., and Pierce, C. H.: Virulence and morphological characteristics of mammalian tubercle bacilli, J. Exper. Med., 1947, *86*, 175.

(2) Dubos, R. J., and Middlebrook, G.: Cytochemical reaction of virulent tubercle bacilli, Am. Rev. Tubere., 1948, *58*, 698.

(3) Paraf, J., Desbordes, J., Fournier, E., and Guyotjeannin, C.: À propos des nouvelles techniques et de differenciation des bacilles tuberculeux, Ann. Biol. Clin., 1952, *10*, 1.

(4) Bloch, H.: Enzymatic characteristics of different mycobacteria, Am. Rev. Tubere., 1950, *61*, 270.

(5) Wilson, F. J., Kalish, C., and Fish, C. H.: Use of oxidation reduction dyes in the determination of virulence of mycobacteria *in vitro*, Am. Rev. Tubere., 1952, *65*, 187.

(6) Singer, J., and Cysner, E.: Urease activity in *Mycobacteriacea*, Am. Rev. Tuberc., 1952, *65*, 779.

(7) Whitehead, J. E. M., Wildy, P., and Engbaek, H. C.: Aryl sulphatase activity of mycobacteria, J. Path. & Bact., 1953, *65*, 451.

(8) Richmond, L., and Cummings, M. M.: An evaluation of methods of testing virulence of acid-fast bacilli, Am. Rev. Tuberc., 1950, *62*, 632.

(9) Hauduroy, P., and Pasternak-Gallia, Y.: Sur une reaction cytochemique en rapport avec la virulence des mycobacteries, Ann. Inst. Pasteur, 1949, *77*, 91.

(10) Viallier, J., Kalb, J. C., and Tiguad, J.: Sur la coloration par le rouge neutre du bacille tuberculeux, Compt. rend. Soc. de Biol., 1950, *144*, 1513.

(11) Morse, W. C., Dail, M. C., and Olitzky, I.: A study of the neutral red reaction for determining the virulence of mycobacteria, Am. J. Pub. Health, 1953, *43*, 36.

(12) Hart, P. D'A, and Rees, R. J. W.: Changes in morphology of an avirulent strain of *Mycobacterium tuberculosis* under the influence of non-ionic surface active agents, J. Gen. Microbiol., 1954, *10*, 150.

(13) Wayne, L. G.: Cultivation and visualization of mycobacteria on molecular filter membranes, Bact. Proc., 1954, p. 92.

(14) Hoyt, A., Smith, C. R., and Gribkoff, G. P.: A filter paper technique for the early detection of microcolonies of tubercle bacilli, Am. Rev. Tuberc., 1954, *70*, 916

(15) Wayne, L. G.: Cultivation and visualization of mycobacteria on molecular filter membranes, J. Bact. (in press).

(16) Dubos, R. J., Pierce, C. H., and Schaefer, W. B.: Antituberculous immunity induced in mice by vaccination with living cultures of attenuated tubercle bacilli, J. Exper. Med., 1953, *97*, 241.

(17) Vorwald, A. J., Dworski, M., and Pratt, P. C.: BCG vaccination in silicosis, Am. Rev. Tuberc., 1954, *69*, 766,

(18) Krasnow, I.: Unpublished observations.

THE DETECTION OF TUBERCLE BACILLI IN MOUTH WASH SPECIMENS BY THE USE OF MEMBRANE FILTER CULTURES[1,2,3]

DAVID E. ROGERS,[4] GEORGE M. COOKE, AND CHARLES E. MEYERS

(Received for publication December 17, 1954)

INTRODUCTION

A simple, reliable method is needed for the culture of tubercle bacilli from respiratory tract secretions. Current methods available for the recovery of tubercle bacilli from patients with suspected or proved pulmonary tuberculosis are often unsatisfactory. Sputum cultures are subject to wide variation in reliability and reproducibility, and depend, in part, upon the training and willingness of patients to produce deep cough specimens. More elaborate cultural procedures utilizing gastric or tracheobronchial specimens require the time of highly trained personnel, equipment not universally available, and are often physically unpleasant to the patients undergoing study. Present cultural methods utilize only a small aliquot of the specimens obtained and do not permit quantitation of the number of tubercle bacilli discharged.

Previous work in this laboratory has indicated that microorganisms in the lower respiratory tract can be isolated from mouth wash specimens (1). The recent development of a new bacteriologic filter, the membrane filter (2), suggested that membrane filter cultures of mouth wash gargle specimens might provide a simple method for the isolation of tubercle bacilli from patients with pulmonary tuberculosis.

The present paper reports the growth characteristics of *Mycobacterium tuberculosis* on membrane filter surfaces and the recovery of tubercle bacilli from patients with pulmonary tuberculosis by the mouth wash–membrane filter technique. While the limitations of this technique have not yet been completely defined, results to date show sufficient promise to justify this preliminary report.

MATERIALS AND METHODS

Membrane filters: Hydrosol assay "Millipore® filters"[5] were used in the studies. These filters are a modification of the "molecular filter" described by Goetz (2) and Goetz and Tsuneishi (3). The filter membrane consists of a homogeneous, screenlike, paper-thin cellu-

[1] From the Naval Medical Research Unit No. 1, and the Naval Biological Laboratory, Department of Bacteriology, University of California, Berkeley, California.

[2] This work was supported in part by a contract between the University of California, Department of Bacteriology, and the Office of Naval Research.

[3] The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1252, U. S. Navy Regulations, 1948).

[4] Present address: The Rockefeller Institute for Medical Research, New York 21, New York.

[5] Manufactured by the Millipore Filter Corporation, Watertown 72, Massachusetts.

lose ester film which is devoid of fibrous structure and which has a high and uniform porosity of approximately 80 per cent of the filter substance. The large number of pores (5×10^7 per sq. cm.) permits the quantitative retention of bacteria at flow rates of 50 to 100 ml. of fluid per minute against differential pressures of 70 mm. of mercury. To facilitate quantitative counts, a grid imprinted on the surface of the filter disc divides the disc surface so that each square represents one-hundredth of the effective filtering surface. Bacterial cells impinged upon the membrane filter surface can be subsequently cultured by diffusion of nutrient when the back surface of the filter is placed in wetting contact with liquid or moist solid nutrient.

Stainless steel filter funnels and bases built in this laboratory were used for filtering procedures (figure 1). A wire mesh grid supported the membrane filter and prevented undue stress on the filter leaf at high-pressure differentials.

The membrane filters were originally sterilized with Carboxide® (ethylene oxide) in a specially adapted autoclave. During the course of the investigations it became apparent that some of the filters so sterilized inhibited microbial growth. Study of the data suggested the possibility of ethylene oxide retention in the filter, and the membrane filters were subsequently sterilized by autoclaving groups of filters in Petri dishes with interposed blotters at 15 psi for ten minutes. The autoclave was exhausted rapidly following sterilization to prevent condensation of moisture on the filter.

Media: Dubos oleic-acid–albumin agar (4) was used for all quantitative studies on the growth of tubercle bacilli on membrane filter surfaces. A small amount of trypan blue was added to facilitate the detection of colonies. In the cultivation of tubercle bacilli on membrane filters prepared from mouth wash specimens from patients, duplicate platings were performed on oleic-acid–albumin agar and Löwenstein medium (Jensen's modification). The Löwenstein medium was prepared in routine fashion, but was inspissated in small (60 mm.) Petri dishes to allow direct plating of the membrane filters.

Liquid Dubos Tween®-albumin medium was used to obtain diffuse cultures for quantitative procedures. Initial attempts to culture tubercle bacilli on the filter surface were performed using the small plastic Petri dishes manufactured by the Millipore Filter Corporation for use with the membrane filter, and liquid oleic-acid–albumin medium was added to the nutrient pad prior to plating. Frequent contamination of membrane filters resulted from this procedure, however, and the water contained in the small amount of nutrient frequently evaporated during the long period of incubation required, despite elaborate techniques designed to maintain humidity. Thus, solid media were used exclusively for membrane filter plating in the present series of experiments.

Cultures: Mycobacterium tuberculosis, strain H37Rv, originally obtained from Dr. S. S. Elberg, Department of Bacteriology, University of California, was used in the initial studies of the growth of tubercle bacilli on the membrane filter. Two recent isolates[7] from patients were also used in studies on the quantitative recovery of tubercle bacilli on the filter surface. All cultures were maintained on Dubos or Löwenstein slants at 4° C. Seven- to fourteen-day liquid Tween®-albumin cultures were used in the preparation of suspensions for filtration.

Enumeration of tubercle bacilli: All culture dilutions and suspensions of tubercle bacilli were made in M/20 phosphate buffered saline, pH 7.0. For work requiring colony counts on the membrane filter the buffered saline was initially filtered through a membrane filter and resterilized in flasks with aluminum foil caps. All other glassware was also rinsed repeatedly with distilled water, and the cotton stoppers were replaced with aluminum foil caps prior to sterilization. This procedure eliminated the small cotton fibrils which tended to accumulate on the filter surface and to obscure the colonies.

Measured aliquots of buffered saline containing tubercle bacilli were filtered through four to ten membrane filters. The quantitative growth of tubercle bacilli on the filter sur-

[6] Union Carbide and Carbon Company, New York, New York.

[7] Obtained through the courtesy of Dr. Ralph Tompsett, Cornell University Medical College, New York, New York.

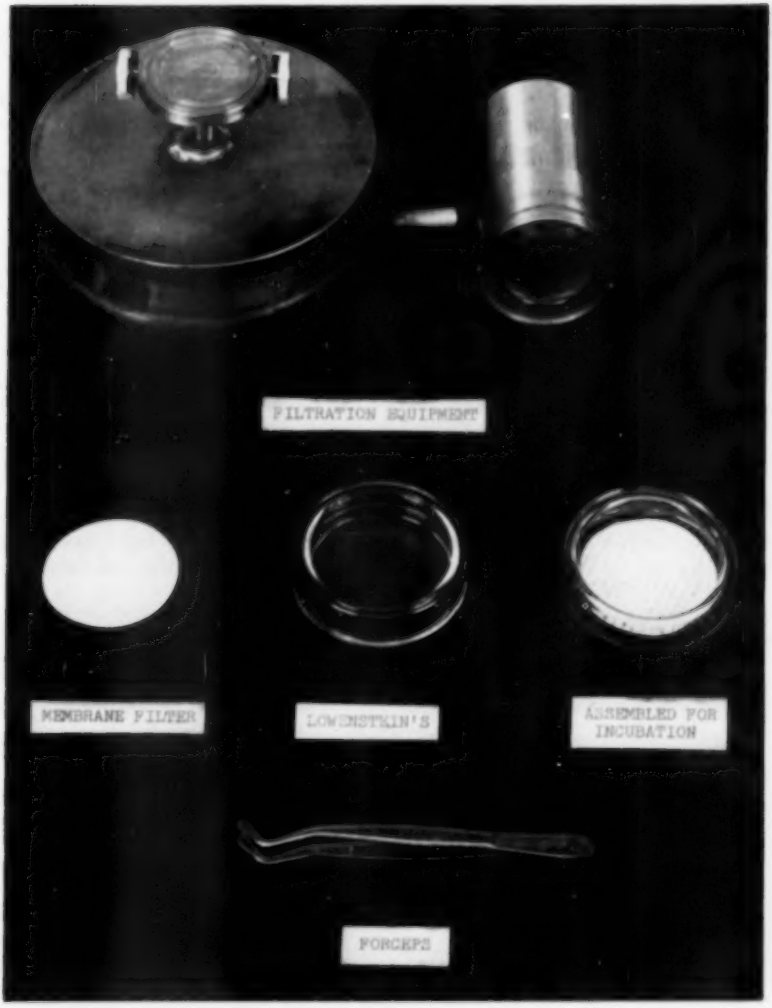


FIG. 1. Filtration equipment used with the membrane filter. The sleeve-like *receptacle* has been removed to reveal the stainless steel screen (120 mesh) which supports the membrane filter during filtration. The membrane filter is 47 mm. in diameter.

face was compared with that obtained on the surface of oleic acid–albumin agar. Colony counts were made on control plates using the technique of Fenner, Martin, and Pierce (5), with the substitution of a blunted 18-gauge needle, a glass adapter, and a 1 ml. rubber bulb, as described by Cohn and Garber (6). Three plates were used for each dilution, and only counts of 30 to 300 colonies per plate were used in final calculations.

It was found that membrane filter cultures were most satisfactory when the plates were inverted and sealed in cans containing moistened cotton to maintain high humidity during

incubation. Any drying of the filter surface resulted in a significant reduction in the number of culturable tubercle bacilli.

Digestion procedures: Three methods were used to reduce mouth wash viscosity and eliminate the normal flora:

1. An equal volume of 4 per cent sodium hydroxide was added to each mouth wash specimen, and the mixture was incubated at 37° C. for fifteen to thirty minutes. Specimens were then neutralized with 1 N hydrochloric acid and filtered.

2. An enzyme solution containing 5 per cent amylase and 20 per cent mycozyme[3] in saline was added to 10 per cent by volume. Digestion was allowed to proceed for thirty minutes at 37° C. prior to filtration.

3. Ten per cent trisodium phosphate was added in equal volumes to mouth wash specimens as described by Corper and Stoner (7). Specimens were incubated at 37° C. for eighteen to twenty-four hours, then neutralized with 1 N hydrochloric acid before filtration.

Clinical specimens: Clinical specimens were obtained from patients at the Veterans Administration Hospital, Livermore, California, through the courtesy of Dr. J. A. Arcellana, Chief of the Laboratory Services. Mouth wash specimens were collected in the morning from recently awakened patients with suspected or proved pulmonary tuberculosis. Simultaneous twenty-four-hour sputum cultures or gastric aspirate cultures were made upon the same patients and were used to check the reliability of membrane filter cultures. Specimens were collected in sterile, screw-capped jars containing 25 ml. of sterile buffered saline. The patient was asked to rinse his mouth and pharynx vigorously for thirty seconds with the entire contents of the jar in a manner similar to gargling. The saline was then expectorated into the jar, and the specimen was returned to the laboratory for digestion. Following digestion, the volume was brought to 100 ml. with buffered saline, and the specimen was filtered in 10 ml. amounts through 10 filters.

Routine cultures of twenty-four-hour sputum collections and gastric aspirates were made on Löwenstein egg medium in the hospital bacteriology laboratory and independently read and recorded. Comparison of routine cultures prepared by the hospital staff and cultures by the mouth wash–membrane filter technique carried out in the laboratory was made following the final reading of the cultures.

RESULTS

Quantitative Recovery of Mycobacterium Tuberculosis on the Membrane Filter Surface

Studies on the quantitative recovery of tubercle bacilli on membrane filters sterilized with ethylene oxide revealed a five- to thirty-fold decrease in the number of culturable tubercle bacilli when compared with control plates. When membrane filters were sterilized in the autoclave, colony counts on membrane filter cultures agreed closely with those obtained on control oleic-acid–albumin plates (table 1). Growth appeared slowly on the filter surface, identifiable colonies becoming apparent in fourteen to twenty-one days on oleic-acid–albumin medium. Colony counts could be made on membrane filter preparations at twenty-one to thirty days. Colonies were visible on oleic-acid–albumin control plates in ten to fourteen days and quantitative counts could be made in sixteen to twenty-one days.

Colony morphology was typical on the filter surface. When plated on oleic-acid–albumin medium, colonies appeared flat and translucent with spreading,

[3] Mycozyme was obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE 1

QUANTITATIVE RECOVERY OF M. TUBERCULOSIS ON MILLIPORE® FILTERS STERILIZED BY ETHYLENE OXIDE OR AUTOCLAVE

Strain *M. tuberculosis*	Membrane Filter Sterilization	Drop Plate Counts Microorganisms per Ml.	Membrane Filter Counts Microorganisms per Ml.
H37Rv	Ethylene oxide	1.8×10^8	3.6×10^7
H37Rv	Ethylene oxide	3.4×10^8	1.2×10^8
H37Rv	Ethylene oxide	9.0×10^7	*8.3×10^6
H37Rv	Ethylene oxide	9.0×10^7	*7.2×10^6
H37Rv	Ethylene oxide	9.0×10^7	*5.7×10^6
H37Rv	Autoclave	2.7×10^7	2.1×10^7
H37, #892	Autoclave	5.8×10^7	5.5×10^7
H37, #961	Autoclave	1.5×10^8	1.6×10^8

* Different filter lot numbers.

Results represent counts from triplicate or quadruplicate plates.

The decrease in the number of culturable microorganisms ranges from five- to thirty-fold when impinged on membrane filters sterilized with ethylene oxide. No loss in culturable microorganisms is apparent on autoclaved membrane filters.

serpentine corded edges (figure 2). On Löwenstein medium, the colonies became typically pigmented, but rarely acquired the heaped, pellicle appearance seen on Löwenstein agar slants (figure 3).

Effects of Digestion on Mouth Wash Viscosity and the Number of Culturable Tubercle Bacilli

Mouth wash specimens were sufficiently viscous to prevent their effective passage through the membrane filter, and normal mouth flora rapidly overgrew the surface despite the addition of inhibitors to the media. To overcome these problems, digestion of mouth wash samples with sodium hydroxide, trisodium phosphate, and an enzyme solution was assessed.

All three digestion procedures effectively reduced the viscosity of mouth wash specimens and allowed rapid filtration of samples. Both sodium hydroxide and trisodium phosphate eliminated normal mouth flora in most instances, while enzymatic digestion had no demonstrable bactericidal effect. In saline suspension, digestion with either sodium hydroxide or trisodium phosphate resulted in a significant loss in the number of culturable tubercle bacilli ranging from forty- to a thousandfold (based on untreated aliquots) and a detectable delay in colony growth. The loss of culturable microorganisms was of the same magnitude when tubercle bacilli were incorporated in mouth wash specimens (table 2). When liquid oleic-acid–albumin cultures containing tubercle bacilli in clumps were digested, the reduction was less apparent (table 2). Under these conditions, it seemed probable that, although many tubercle bacilli in each clump were rendered nonviable, cells sufficient in number to initiate growth remained protected within a clump. Enzymatic digestion had no demonstrable effect on either the number of tubercle bacilli or the speed of visible colony growth.

While enzymatic digestion appeared to be the method of choice in maintaining

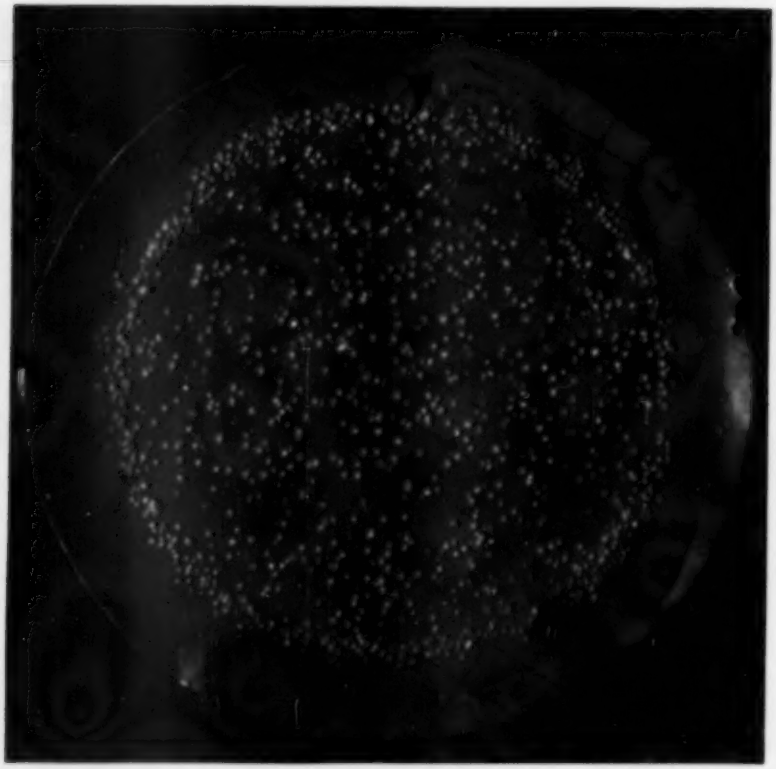


FIG. 2. The growth of *M. tuberculosis* (H37Rv) on the membrane filter. Twenty-five-day culture on Dubos solid medium. Original inoculum obtained from liquid oleic-acid-albumin culture. (Magnification 2.5×)

viable tubercle bacilli, it has not been possible to date to develop satisfactory media for the selective elimination of normal oral flora. Reduction of sodium hydroxide time to fifteen minutes resulted in less destruction of tubercle bacilli, eliminated oral flora in most instances, and reduced viscosity sufficiently to allow prompt filtration (table 2). Sodium hydroxide was consequently used in the treatment of the majority of clinical specimens.

Recovery of Tubercle Bacilli from Patients by the Mouth Wash–Membrane Filter Technique

Specimens from 134 patients under treatment at the Veterans Administration Hospital, Livermore, California, were examined by the mouth wash–membrane filter culture method, and the results were compared with those obtained by routine culture (table 3). Mouth wash specimens were collected from 43 patients who completed twenty-four-hour sputum collections.

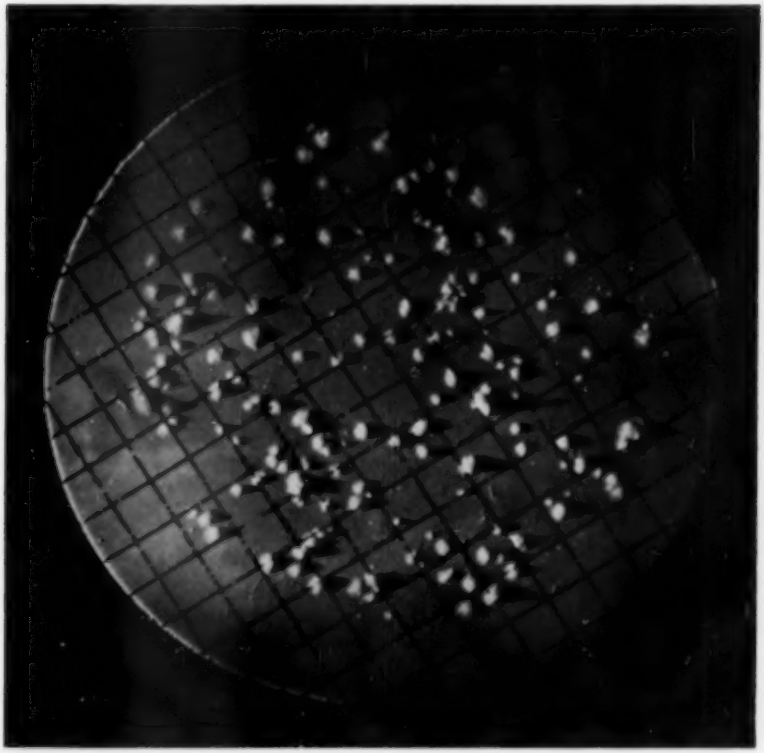


FIG. 3. The growth of *M. tuberculosis* on a membrane filter prepared from the mouth wash specimen of a tuberculous patient. Twenty-five-day growth on Löwenstein medium. Note the typical colonial appearance and the uniform distribution of colonies found in such filtration procedures (Magnification 2.5×)

Twenty-three specimens were positive for *M. tuberculosis* on routine culture and 22 positive cultures were obtained by the mouth wash–membrane filter technique. Discrepant findings in specimens from 12 patients are indicated in table 4. Five patients with specimens positive by mouth wash–membrane filter culture had negative routine sputum cultures. The mouth wash–membrane filter cultures from 5 patients showing positive twenty-four-hour sputum cultures were contaminated with fungi and could not be assessed, while the mouth wash–membrane filter cultures were negative in one patient from whose sputum tubercle bacilli could be cultured. This resulted in 6 failures to obtain positive mouth wash–membrane filter results in the face of twenty-four-hour sputum cultures positive for tubercle bacilli.

Mouth wash specimens were collected from 91 patients with negative sputum cultures who were undergoing gastric aspiration for culture (table 3). One mouth wash specimen was positive for *M. tuberculosis* on membrane filter culture.

TABLE 2

EFFECTS OF DIGESTION ON THE QUANTITATIVE RECOVERY OF CULTURABLE TUBERCLE BACILLI

Comparative Effects of Sodium Hydroxide, Trisodium Phosphate, and Enzyme Solution on Microorganisms Growing in Diffuse Culture

Control Plates Microorganisms per Ml.	4 Per Cent Sodium Hydroxide 30 Minutes Microorganisms per Ml.	10 Per Cent Trisodium Phosphate 18 to 24 Hours Microorganisms per Ml.	10 Per Cent Enzymes* 30 Minutes Microorganisms per Ml.
4×10^7	1×10^4		
1.7×10^8	7×10^5		
1.2×10^8	4×10^6†	3.2×10^5†	
1.6×10^8	5.2×10^5†	1.0×10^6†	•
1.9×10^7	4.4×10^6‡	9.9×10^6	1.9×10^7
Effect of Sodium Hydroxide on Microorganisms Growing in Clumped Cultures			
7×10^5	8×10^6		
6×10^6	1.7×10^5		

* 5 per cent amylase, 20 per cent myceozyme.

† Tubercle bacilli incorporated in mouth wash prior to digestion.

‡ Fifteen-minute sodium hydroxide digestion.

There is a forty- to thousandfold reduction in culturable tubercle bacilli following digestion with sodium hydroxide or trisodium phosphate. Less reduction (nine- to forty-fold) occurs when the inoculum is obtained from clumped (oleic-acid–albumin) cultures. No reduction in culturable microorganisms results from enzymatic digestion.

TABLE 3

COMPARISON OF THE RECOVERY OF M. TUBERCULOSIS BY MOUTH WASH–MEMBRANE FILTER CULTURES AND BY ROUTINE CULTURAL TECHNIQUES

Type of Culture Specimens Obtained	Number of Patient Specimens Examined	Positive for *M. tuberculosis* by Mouth Wash-Membrane Filter Culture	Positive for *M. tuberculosis* by Routine Culture
Sputum and mouth wash–membrane filter cultures	43	22	23
Gastric aspirates and mouth wash–membrane filter cultures (from patients with negative sputum cultures)	91	1	3
Totals	134	23	26

Routine gastric aspirate culture revealed *M. tuberculosis* in 3 specimens. The filter cultures prepared on one patient reported positive for *M. tuberculosis* by gastric aspirate culture were contaminated with fungi.

Appearance of Colonies of M. Tuberculosis on Membrane Filter

Colonies of tubercle bacilli obtained from patients appeared typical on the membrane filter surface (*see* figure 3) and could be detected most easily using

TABLE 4

DISCREPANT CULTURAL RESULTS BY MOUTH WASH–MEMBRANE FILTER CULTURE AND ROUTINE CULTURE OF SPUTUM AND GASTRIC ASPIRATES

Specimens positive for *M. tuberculosis* on membrane filter culture, negative on sputum culture	5
Specimens positive for *M. tuberculosis* on sputum culture, negative on membrane filter culture	6*
Specimens positive for *M. tuberculosis* on gastric aspirate culture, negative on membrane filter culture	2†

* Membrane filter cultures from 5 patients with sputum positive for *M. tuberculosis* on culture were contaminated with fungal overgrowth and could not be read.

† Membrane filter cultures from one patient were contaminated.

Galley 2X

incident lighting and a low-power dissecting microscope. Positive colonial identification was made in periods ranging from fourteen to sixty days. Growth, in general, was slower on the membrane filter surface than it was on routine culture media. Many patients were receiving antituberculous drug therapy, however, and colony growth was slow by all methods of culture.

DISCUSSION

The present study has shown that tubercle bacilli can be collected and quantitatively cultured on the surface of Millipore® filters. Utilizing mouth wash specimens from tuberculous patients, positive cultures were obtained from the majority of patients with sputum cultures positive for tubercle bacilli. An insufficient number of patients with negative sputum and positive gastric aspirate cultures for tubercle bacilli have been studied to date to make possible any statement regarding the sensitivity of the procedure under these circumstances.

Colony growth was significantly slower on the membrane filter surface than it was on the surface of routine plating media. Recent information from Goetz (8) suggests that this difficulty may be prevented by increasing the strength of medium constituents to overcome the diluting effect of atmospheric water absorption by the exposed membrane filter surface.

The use of alkali digestion results in the destruction of the majority of tubercle bacilli obtained in mouth wash specimens as well as a significant delay in colony appearance. These findings have been borne out by a recent extensive study of the lethal effects of concentrating agents on tubercle bacilli by Gray, Clark, and Johnson (9). Nevertheless, alkali digestion was found necessary to reduce mouth wash viscosity for filtration and to eliminate normal mouth flora. Sodium hydroxide was found to be the most satisfactory agent for digestion, resulting in the least destruction of tubercle bacilli, but fungal contamination of membrane filter cultures was not satisfactorily eliminated. Since the completion of this work, a report by Hirsch (10) has indicated that benzalkonium chloride (Zephiran®) eliminates normal sputum contaminants without significant destruction of tubercle bacilli. It is planned to continue studies using enzymatic digestion in conjunction with benzalkonium chloride decontamination in the preparation of mouth wash specimens for culture.

Because of the delay in diagnosis inherent in the slow cultural growth of tubercle bacilli, the use of the membrane filter as a collecting device, and subsequent direct staining of tubercle bacilli impinged on the filter, is under study. A recent modification of the neutral red staining technique reported by Wayne (11)[9] allows the detection of microcolonies of tubercle bacilli on the membrane filter surface after four to six days of incubation. Its use in conjunction with the mouth wash culture technique for more rapid diagnostic identification is contemplated.

The ability of membrane filters to transmit large quantities of fluid at high rates of speed permits the concentration of small numbers of microorganisms contained in large volumes. Thus, membrane filter cultures may prove of value in clinical situations where bacteriologic diagnosis is at present difficult. The detection of tubercle bacilli (or other microorganisms) in urine, or in pleural, pericardial, or cerebrospinal fluid may be facilitated by the use of membrane filter cultures, and such studies are now in progress.

Microorganisms may also be separated from possible inhibitory substances contained in body fluids by washing of the filter prior to plating. Such procedures may allow the recovery of microorganisms which are unable to multiply under ordinary cultural conditions.

Studies to be reported have indicated that microorganisms with widely differing biochemical requirements can be cultured quantitatively on the membrane filter surface, and that colony growth is more rapid on the filter surface than on ordinary plating media. Colonies of staphylococci, *Klebsiella*, and *Salmonella* are visible on membrane filter cultures in six to twelve hours in contrast to the twelve to twenty-four hours ordinarily required for visible surface growth (12). Swifter bacteriologic methods are needed for diagnosis in many clinical situations to allow more rational and precise use of the powerful antimicrobials now available. The use of membrane filter cultures is thus being explored in other clinical situations where specific cultural diagnosis may be aided by the unique characteristics of the membrane filter.

SUMMARY

A mouth wash gargle technique has been used in conjunction with membrane filter cultures for the recovery of tubercle bacilli from patients with pulmonary tuberculosis.

Cultures positive for tubercle bacilli have been obtained by this technique in the majority of patients with positive sputum cultures.

Further studies to assess the sensitivity of the mouth wash–membrane filter culture technique in patients with negative sputum in whom gastric or tracheobronchial aspirates must be obtained are in progress.

The membrane filter culture technique described has possible application in other clinical situations where bacteriologic diagnosis is difficult with routine cultural procedures.

[9] Also see KRASNOW, I., WAYNE, L. G., AND SALKIN, D., in this issue of the REVIEW, page 361—*The Editor*

SUMARIO

El Descubrimiento de Bacilos Tuberculosos en Ejemplares de Lavados Bucales con el Uso de Cultivos Filtrados por Membranas

Para la obtención de bacilos tuberculosos de enfermos que tenían tuberculosis pulmonar se usó una técnica de gargarismos-lavados bucales unida a filtración de cultivos a través de una membrana.

Con esa técnica obtuviéronse cultivos positivos para bacilos tuberculosos en la mayoría de los enfermos cuyo esputo era positivo en cultivos.

Ya se han emprendido estudios ulteriores para determinar la sensibilidad de la técnica de cultivos filtrados por membrana y lavados bucales en los enfermos que tienen esputo negativo y en quienes hay que obtener aspirados gástricos o tráqueobronquiales.

La técnica aquí descrita de cultivos filtrados por membrana tiene posible aplicación a otras situaciones clínicas en las que el diagnóstico bacteriológico resulta difícil con los procedimientos culturales de costumbre.

RESUME

Découverte du bacille tuberculeux dans les prélèvements de lavage buccal par la méthode des cultures sur membrane filtrante

Une technique de lavage buccal par gargarisme a été utilisée en association avec la culture sur membrane filtrante pour l'isolement des bacilles tuberculeux chez les malades atteints de tuberculose pulmonaire.

Des cultures positives pour le BK ont été obtenues par cette technique chez la majorité des malades dont la culture de l'expectoration était positive.

D'autres recherches sont en cours pour déterminer la sensibilité de la technique lavage buccal- culture sur membrane filtrante quand il s'agit de malades ayant des crachats négatifs et qu'il est nécessaire d'obtenir des spécimens gastriques ou trachéo-bronchiques par aspiration.

La technique de culture sur membrane filtrante décrite a des applications possibles dans d'autres conditions cliniques où le diagnostic par les méthodes culturales classiques est difficile.

REFERENCES

(1) Ostrom, C. A., and Wolochow, H.: Unpublished data, 1950.
(2) Goetz, A.: Fiat Final Report 1312, Joint Intelligence Objectives Agency, U. S. Department of Commerce, Washington, D. C., 1947.
(3) Goetz, A., and Tsuneishi, N.: Application of molecular filter membranes to the bacteriological analysis of water, *J.* Am. Water Work Assoc., 1951, *43*, 943.
(4) Dubos, R. J., and Middlebrook, G.: Media for tubercle bacilli, Am. Rev. Tubere., 1947, *56*, 334.
(5) Fenner, F., Martin, S. P., and Pierce, C. H.: The enumeration of viable tubercle bacilli in cultures and infected tissues, Ann. New York Acad. Sc., 1949, *52*, 751.
(6) Cohn, T., and Garber E.: Personal communication.
(7) Corper H. J., and Stoner, R. E.: An improved procedure for the diagnostic culture of tubercle bacilli, J. Lab. & Clin. Med., 1946, *31*, 1364.
(8) Goetz, A.: Personal communication, 1954.
(9) Gray, D. F., Clark, B. L., and Johnson, W. E.: Detection of small numbers of tubercle bacilli in diagnosis: The lethal action of concentrating agents, Am. Rev. Tubere., 1954, *69*, 991.
(10) Hirsch, J. G.: The resistance of tubercle bacilli to the bactericidal action of benzalkonium chloride (Zephiran®), Am. Rev. Tubere., 1954, *70*, 312.
(11) Wayne, L. G.: Technique for cultivation of mycobacteria on molecular filter membranes, J. Bact. (in press).
(12) Rogers, D. E.: Unpublished observations, 1954.

CHARCOAL AGAR MEDIA FOR THE CULTIVATION OF MYCOBACTERIUM TUBERCULOSIS[1]

JOSEPH W. WHALEN[2] AND W. L. MALLMANN

(Received for publication September 27, 1954)

INTRODUCTION

Mycobacterium tuberculosis has been found to be highly susceptible to the toxic effect of many substances customarily present in trace amounts on culture media. Long-chain fatty acids and lipoids present in agar, cotton, or improperly cleaned culture tubes inhibit the growth of small inocula. Larger numbers of tubercle bacilli probably adsorb sufficient lipid to permit unimpaired multiplication of some of them. Drea and Andrejew (1) demonstrated that starch, potato extract, and egg-yolk components apparently also will reduce the inhibition. Davis and Dubos (2) found that bovine serum albumin fraction V in culture media combines with fatty acids toxic to tubercle bacilli to permit and enhance the growth of small numbers of the microorganisms. Youmans and Youmans (3) subsequently reported greater growth stimulation of *M. tuberculosis* by bovine serum than by bovine fraction V albumin. Ley and Muller (4) found that agar contains a fatty acid-like substance which is inhibitory for *Neisseria gonorrhoeae*; the addition of starch or charcoal to the medium eliminates the toxicity, probably through adsorption of this substance. Pollock (5) reported that either blood albumin or charcoal in a culture medium will neutralize the toxic action of fatty acids for *Hemophilus pertussis*. Powell, Culbertson, and Esminger (6) found that *H. pertussis* will grow luxuriantly on a charcoal agar medium. Stonebrink (7) compared a dextrinized starch–activated charcoal egg medium with that of Löwenstein and found that, with bovine tubercle bacilli, growth was speeded 40 per cent on the former medium.

In view of these findings, a controlled study of the effects of different grades of activated charcoal on the growth of recently isolated strains of tubercle bacilli of human and avian origin seemed warranted. If one or more of these charcoals produced a stimulatory effect comparable to that of blood component media enrichments, a comparison of media containing them with the Petragnani egg medium could be made. The latter medium in previous comparative studies in this laboratory had been found to be the most satisfactory of the classical egg media for the cultivation of *M. tuberculosis*.

MATERIALS AND METHODS

Graded inocula of tubercle bacilli were prepared according to the method of Youmans and Karlson (8) in the following fashion: Three strains of *M. tuberculosis* var. *hominis*, recently isolated, were mixed and standardized, using Hopkins centrifuge tubes to deter-

[1] From the Department of Bacteriology and Public Health, Michigan State College, East Lansing, Michigan.

[2] Medical Research Fellow of the National Tuberculosis Association.

mine the initial mass of organisms. All strains had been cultivated on the basal medium enriched with bovine albumin fraction V for twelve to sixteen days. Two strains of *M. tuberculosis* var. *avium* were similarly mixed and standardized. From these suspensions, dilutions were made with 0.01 M phosphate buffer solution, pH 7.0, containing 0.2 per cent Triton A-20®, so that the inocula to be employed contained, respectively: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} mg. of tubercle bacilli, wet weight. Using the Youmans and Youmans (9) procedure, these concentrations permitted calculation of the generation times of the organisms on each of the media studied.

The basal medium utilized was composed of the following ingredients in grams per liter: proteose peptone No. 3 (Difco), 2.0; Na_2HPO_4, 2.5; KH_2PO_4, 1.0; $CuSO_4$, 0.0001; Co $3O_4$, 0.0001; ferric ammonium citrate, 0.1; glucose, 7.5; glycerol, 2.5; Savita®[5], 2.0; Triton A-20®, 0.5; and agar, 15.6. Four grams of each of the following grades of Nuchar® activated charcoal[6], were added to liter portions of this basal medium: C-115-N, C-190-N, WA, and Aqua A. The final pH of all of the media was 6.8. The media were tubed in 5 ml. amounts in screw-capped tubes, and were sterilized at 121°C. for fifteen minutes. While the media were cooling, the tubes were placed in a slanted position.

Before utilization of the diluted inocula, one tube of each of the media was seeded with 10^{-1} mg. of a recently isolated human strain of *M. tuberculosis*. Multiplication of this strain was noted within two days' incubation. This established that each of the charcoal media would support growth.

In generation time studies, two tubes of each medium were inoculated with each of the individual concentrations of tubercle bacilli. All cultures were incubated at 37.5°C. and were examined at daily intervals macroscopically. Evidence of growth was confirmed by preparation of slide films stained with the Ziehl-Neelsen technique, and subsequent microscopic examination. Several separate trials were made of each of the media, using the actively growing human and avian tubercle bacilli.

Ethyl violet 6B was subsequently added to the best charcoal-enriched media in concentrations ranging from 1:200,000 to 1:1,300,000. Litsky, Mallmann, and Fifield (10) found that this dye, in proper concentration, will inhibit Gram-positive bacteria, but exhibits less toxicity for Gram-negatives than do crystal violet and brilliant green in comparable concentrations. Whalen and Mallmann (11) found that ethyl violet manifests less toxicity for *M. tuberculosis* than does malachite green in concentrations having equivalent inhibitory effect on Gram-positive bacteria.

Media utilized in the isolation of *M. tuberculosis* from sputum contained 100 units of potassium penicillin G and 100γ of cycloheximide (Actidione®) per ml.

The specimens of sputum were digested with 3 per cent sodium hydroxide for one-half hour and neutralized with 3 per cent hydrochloric acid. Concentration was effected by centrifugation at 2,000 r.p.m. for twenty minutes. The sediments were used to prepare smears for microscopic examination and for inoculation of the media, four loopfuls being used for each tube. Two tubes of each medium were inoculated and subsequently incubated at 37.5°C. During incubation the cultures were examined macroscopically at intervals of two days. All colonies were checked microscopically, using the Ziehl-Neelsen stain. After forty-two days a final reading was made for all cultures and they were discarded. The concentrate smears, two of which were made for each sediment, were also Ziehl-Neelsen stained and examined for at least twenty minutes.

RESULTS

The generation times in hours of the human and avian tubercle bacilli when growing on five types of media are presented in table 1. The five media were the basal medium, the charcoal media, and the basal medium enriched with

[5] A yeast-vegetable extract with ferric citrate.

[6] Supplied by Mr. Irving Dahlijelm, Department of Bacteriology and Public Health, Michigan State College, East Lansing, Michigan.

TABLE 1

THE EFFECT OF FOUR ACTIVATED CHARCOALS AND THREE PROTEIN ENRICHMENTS ON THE RATE OF GROWTH OF RECENTLY ISOLATED STRAINS OF HUMAN AND AVIAN TUBERCLE BACILLI

Medium	Trial	*M. Tuberculosis* var. *Hominis*		*M. Tuberculosis* var. *Avium*	
		Generation Time in Hours	Per Cent Stimulation over Basal Medium	Generation Time in Hours	Per Cent Stimulation over Basal Medium
Basal medium	1	28.8		26.4	
	2	28.8		26.4	
	3	28.8			
Basal + C-190-N	1	22.8	20.8	22.8	13.6
	2	22.8	20.8	22.8	13.6
	3	22.8	20.8		
Basal + C-115-N	1	24.0	16.7	24.0	9.1
	2	24.0	16.7	22.8	13.6
	3	24.0	16.7		
Basal + WA	1	27.6	4.1	24.0	9.1
	2	27.6	4.1	25.4	3.8
	3	27.6	4.1		
Basal + Aqua	1	28.8		25.4	3.8
	2	28.8		25.4	3.8
Basal + oleic albumin	1	22.8	20.8	24.0	9.1
	2	22.8	20.8	22.8	13.6
	3	24.0	16.7		
Basal + bovine albumin	1	21.7	24.6	20.5	22.3
	2	21.7	24.6	19.3	26.9
	3	22.8	20.8		
Basal + human serum	1	20.8	20.8	20.5	22.3
	2	22.8	20.8	21.7	17.8

0.2 per cent bovine albumin, 10 per cent human serum, and 10 per cent Dubos oleic albumin complex (Difco). The percentage of stimulation of the rate of growth on each medium over that on the basal medium is also given for both varieties of tubercle bacilli.

Growth was most rapid on the bovine albumin-enriched medium. Extreme differences in effect on the growth of tubercle bacilli were noted with the different types of activated carbons; C-190-N stimulated growth of the human strain 20.8 per cent, as did the human serum medium. The C-115-N charcoal medium accelerated the generation time 16.7 per cent, whereas the WA charcoal medium produced only 4.1 per cent stimulation over the basal medium, and Aqua A exhibited no stimulation. Although generation times were less regular, the avian tubercle bacilli manifested a similar pattern of response to the four charcoals, C-190-N again proving to be the most effective in stimulating the rate of growth. With both varieties of *M. tuberculosis*, the oleic albumin enrichment was nearly as effective as C-190-N. In the first two trials with human strains, the generation times (22.8 hours) were identical on the two media.

Twenty-six three-day pooled sputum specimens were cultured on Petragnani

TABLE 2

RESULTS OF CULTURING 26 THREE-DAY POOLED SPUTUM SPECIMENS, IN 4 OF WHICH ACID-FAST BACILLI WERE DETECTED BY MICROSCOPIC EXAMINATION

Medium	Ethyl Violet Content	Average Time in Days Growth First Appeared	Positive for *M. Tuberculosis*	Contaminated	Overgrown with Contaminants
Charcoal (C-115-EV)	1:1,300,000	12	7	18	5
Charcoal (C-190-EV)	1:1,300,000	10	10	15	5
Petragnani	None	20	6	12	3

medium, and on the charcoal media C-190-N and C-115-N. The charcoal media contained 1:1,300,000 ethyl violet, as well as the concentrations of penicillin and cycloheximide previously noted.

Table 2 records the number of isolations and the average time required for detection of *M. tuberculosis* on the three media. In addition, the table includes the contamination encountered in culturing the 26 specimens, in 4 of which acid-fast bacilli morphologically identical to tubercle bacilli were found by microscopic examination. The Petragnani medium yielded 6 cultures of *M. tuberculosis* as compared with 7 for the charcoal medium (C-115-EV). However, the least contamination was experienced with the Petragnani medium. It was possible to detect colonies of *M. tuberculosis* on some cultures which were contaminated but not overgrown.

The excessive contamination of the experimental media was considered to be probably due to reduction in active ethyl violet concentration through its adsorption by the charcoal. This possibility led to a study of generation times of human tubercle bacilli on these two media, as well as on different preparations of C-190-EV containing: 1:200,000, 1:400,000, 1:600,000, 1:1,000,000 and 1:1,200,000 ethyl violet. This experiment was done in order to determine the highest concentration of ethyl violet which could be used in this medium without hindering multiplication of tubercle bacilli.

As shown in table 3, the generation times for all concentrations of ethyl violet in the charcoal (C-190-EV) medium were identical until, at 1:400,000, it rose from 22.8 to 24.0 hours. In the medium containing 1:200,000 ethyl violet, the time greatly increased to 31.2 and 32.6 hours. Therefore, this medium was not included in the study of these media in the culture of three-day pooled specimens of sputum, the results of which are presented in table 4.

In culturing 42 specimens, 11 isolations of tubercle bacilli were obtained with the charcoal C-190-EV medium, which contained 1:400,000 ethyl violet, as compared with 8 for the Petragnani medium, and the number of contaminated cultures was less on the charcoal than in any other medium. Fewer cultures of tubercle bacilli were obtained with the other media, probably due to a greater number of tubes being partially or completely overgrown with contaminants. As on the Petragnani medium, tubercle bacilli were isolated in some instances on all charcoal media, although other microorganisms had likewise produced colonies on the plate.

TABLE 3

THE RATE OF GROWTH OF RECENTLY ISOLATED STRAINS OF M. TUBERCULOSIS VAR. HOMINIS ON CHARCOAL AGAR MEDIA WITH INCREASING CONCENTRATIONS OF ETHYL VIOLET

Grade of Charcoal in Medium	Ethyl Violet Content	Trial	Generation Time in Hours
C-115-EV	1:1,300,000	1	25.4
		2	25.4
C-190-EV	1:1,300,000	1	22.8
		2	22.8
C-190-EV	1:1,200,000	1	22.8
		2	22.8
C-190-EV	1:1,000,000	1	22.8
		2	22.8
C-190-EV	1:800,000	1	22.8
		2	22.8
C-190-EV	1:400,000	1	24.0
		2	24.0
C-190-EV	1:200,000	1	31.2
		2	32.6

TABLE 4

RESULTS OF CULTURING 42 THREE-DAY POOLED SPUTUM SPECIMENS, IN 3 OF WHICH ACID-FAST BACILLI WERE DETECTED BY MICROSCOPIC EXAMINATION

Medium	Ethyl Violet Content	Positive for *M. Tuberculosis*	Contaminated	Overgrown with Contaminants
Charcoal (C-190-EV)	1:400,000	11	12	2
Charcoal (C-190-EV)	1:600,000	8	17	3
Charcoal (C-190-EV)	1:800,000	7	25	8
Charcoal (C-190-EV)	1:1,000,000	3	33	14
Charcoal (C-190-EV)	1:1,200,000	2	38	13
Petragnani	None	7	31	14

Ethyl violet in a concentration of 1:400,000 was incorporated in the three other charcoal media previously studied to determine the effect of this dye in the presence of C-115-N, WA, and Aqua A on the generation times of a mixture of two newly isolated strains of human tubercle bacilli. These charcoal media were compared with the basal medium and with the basal medium enriched with the substances previously studied. The generation time of these organisms was 27.6 hours on the charcoal (C-190-EV) medium, as opposed to 24.0 hours for the human strains previously used in the study of this medium. However, the findings with the other media corresponded to the previous results in that they stimulated growth to approximately the same extent over the generation time on the enriched medium.

The experimental charcoal medium (C-190-EV) containing 1:400,000 ethyl-violet was compared in routine culture of 70 specimens of sputum, gastric washings, and other pathologic material. Seven of these specimens were positive for

acid-fn oast bacilli microscopic examination. Eighteen isolations of *M. tuberculosis* were obtained on the charcoal medium; while, on Petragnani, 12 specimens were positive for tubercle bacilli. Fourteen charcoal (C-190-EV) medium cultures showed some contamination and 5 were overgrown with the contaminating microorganisms. On Petragnani medium, 26 were partially contaminated and 6 were overgrown. Thus, a greater recovery of tubercle bacilli with fewer contaminated cultures was achieved using the charcoal experimental medium.

DISCUSSION

The Nuchar® grades C-190-N and C-115-N of activated charcoal both stimulated growth of tubercle bacilli sufficiently to be utilized in culture media as substitutes for human and bovine serum or albumin. The charcoal grade C-190-N proved superior in this respect. Use of this activated carbon simplifies the preparation of culture media since, unlike the other enrichments studied, it can be added before sterilization by autoclaving. Thus, a saving in time is effected and the danger of accidental contamination during preparation is reduced. The penicillin cycloheximide (Actidione)® inhibitory mixture is not as likely to contain viable bacteria before its addition to the medium. The greatest stimulatory effect was obtained through the addition of bovine albumin to the basal medium. The charcoal C-190-N enhanced growth nearly as effectively as bovine albumin, however, and was approximately equivalent to human serum with respect to stimulatory effect.

The activated charcoals used in the various media are purified products of cellulose manufacture. Each grade is designed for a particular function. Thus, it is not unexpected that differences in effectiveness in promoting growth of *M. tuberculosis* might be found in this study. The C-115-N and C-190-N grades of charcoal will adsorb fat and fatty acids more effectively than will the grades WA and Aqua A (12). The WA grade charcoal has a low affinity for dyes, which is reflected in the greater effectiveness of more dilute concentrations of ethyl violet in the WA charcoal medium in preventing the growth of contaminants.

The writers believe that the activated charcoals act as protective agents, as do starch and blood and egg constituents, adsorbing toxic substances present in trace amounts in the medium. Whether they serve other purposes in the medium is not known at present. Oxygen bound to the surface of the charcoal particles may be available for utilization by the highly aerobic tubercle bacillus.

In culturing pathologic material, growth of *M. tuberculosis* on the charcoal C-190-EV medium was much more abundant at the conclusion of the 42-day incubation period than on the Petragnani medium.

The 1:400,000 concentration of ethyl violet used in the final charcoal (C-190-EV) medium is greater than that found necessary to inhibit contaminants in agar enriched with bovine albumin or oleic albumin. In these media, this amount of dye was found to be toxic for the tubercle bacillus. In the presence of the charcoals, however, a portion of the added ethyl violet is rendered inactive, probably through adsorption.

The concentrations of penicillin and cycloheximide (Actidione®) were deter-

mined through previous study in other experimental media. The presence of ethyl violet, as used in the media, does not prevent growth of streptococci and fungi. Use of penicillin effectively reduces contamination by the former. Cultures inoculated with sputum from patients receiving streptomycin therapy have been found to be frequently contaminated with fungi, especially those of yeastlike morphology. Cycloheximide has reduced this contamination extensively. The charcoal agar media containing these agents and used in the comparative studies were contaminated more frequently than had been the case in the past when penicillin and cycloheximide were incorporated in ethyl violet agar media enriched with bovine albumin. Perhaps the activated carbon also reduced the activity of these agents.

It was anticipated that the avian strains of *M. tuberculosis* would grow more rapidly on all media than did those of the human variety. However, little difference in the generation time was noted. Perhaps the fact that one of the two strains used had been very recently isolated was responsible for the deviation from behavior previously experienced with such strains. Final growth on all media, however, was more luxuriant with the avian bacilli.

SUMMARY

The growth stimulation of recently isolated strains of *Mycobacterium tuberculosis* produced by bovine albumin, oleic albumin, human serum, and four grades of activated charcoal was determined. Bovine albumin produced the greatest stimulation. The effect of charcoal C-190-N was equivalent to human serum and oleic albumin. Two other charcoals stimulated growth to a lesser extent.

Studies of generation times of tubercle bacilli on agar media containing charcoal C-190-N and use of these media for primary isolation of *M. tuberculosis* established that 1:400,000 is the optimum selective concentration of ethyl violet 6B. In culturing pathologic material, the charcoal (C-190-N) agar containing ethyl violet, penicillin, and cycloheximide (Actidione®) was less frequently contaminated than the Petragnani medium. A greater and more rapid recovery of *M. tuberculosis* was obtained with this charcoal medium.

Activated charcoal probably adsorbs substances toxic to tubercle bacilli. The medium is economically and easily prepared; sterilization is by autoclaving.

SUMARIO

Los Medios de Agar y Carbón Vegetal para el Cultivo del Mycobacterium Tuberculosis

Quedó demostrada la excitación del desarrollo de cepas recién aisladas del *Mycobacterium tuberculosis* producida por la albúmina bovina, la albúmina oleica, el suero humano y cuatro clases de carbón vegetal activado. La albúmina bovina produjo la mayor excitación. El efecto del carbón vegetal C-190-N fué equivalente al del suero humano y de la albúmina oleica. Los carbones vegetales C-115-N y WA también excitaron el desarrollo, pero en menor grado.

Los estudios de los tiempos de generación de los bacilos tuberculosos en medios de agar que contenían C-190-N y varias concentraciones de violeta de etilo 6B, así como el cultivo de esputos combinados de tres días en dichos medios, establecieron que una concentración al 1:400,000 de ese colorante no afecta apreciablemente la velocidad del desarrollo, pero sí ayuda a inhibir la proliferación de bacterias inconvenientes. El agar C-190-N, que contenía violeta de etilo al 1:400,000, 100 unidades de penicillina por ml. y 100 γ de Actidione® por

ml., al cultivar material patológico, resultó estar contaminado menos frecuentemente que el medio de Petragnani. Con este medio de carbón vegetal se obtuvo una cosecha mayor y más rápida del *M. tuberculosis*.

El carbón vegetal activado adsòrbe probablemente substancias tóxicas para los bacilos tuberculosos. El medio es fácil de preparar, dado que la esterilización es al autoclave.

RESUME

Milieu au charbon-agar pour la culture du Mycobacterium tuberculosis

Démonstration de la stimulation de la croissance de souches fraîchement isolées de *M. tuberculosis* sur milieux albumine bovine, albumine oléique, sérum humain et quatre catégories de charbon activé. L'albumine bovine procure la stimulation la plus marquée. L'action du charbon est égale à celle du sérum humain et de l'albumine oléique. Les charbons C-115-N et WA stimulent également la croissance, mais à un moindre degré. Aqua A ne modifiait pas le temps de reproduction obtenu avec le milieu de base.

La recherche du temps de reproduction des B.K. sur le milieu agar renfermant le C-190-N et le violet-éthyle 6B à diverses concentrations, de même que la culture sur ces milieux de crachats recueillis pendant trois jours, ontdémontré que ce colorant, à la concentration de 1/400.000, n'influence pas le taux de croissance de façon appréciable, mais facilite l'inhibition de la croissance des bactéries indésirables. L'agar C-190-N, renfermant 1/400.000 de violet-éthyle, 100 unités de pénicilline et 100 γ d'Actidione® par ml., utilisé pour cultiver l'échantillon pathologique, a été trouvé moins fréquemment contaminé que le milieu Pétragnani. Ce milieu au charbon a permis d'obtenir le *M. tuberculosis* en plus grande quantité et plus rapidement.

Le charbon activé adsorbe probablement substances toxiques pour le B.K.. La préparation de ce milieu est facile, car la stérilisation se fait à l'autoclave.

REFERENCES

(1) Drea, W. F., and Andrejew, A.: The Metabolism of the Tubercle Bacillus, Charles C Thomas, Springfield, Illinois, 1953, p. 95.

(2) Davis, B. D., and Dubos, R. J.: Interaction of serum albumin, free and esterified oleic acid and lipase in relation to cultivation of the tubercle bacillus, Arch. Biochem., 1946, *11*, 201.

(3) Youmans, A. S., and Youmans, G. P.: The effect of bovine plasma fractions on the growth of *Mycobacterium tuberculosis* var. *hominis*, J. Bact., 1950, *60*, 561.

(4) Lee, H. L., and Muller, J. H.: On the isolation from agar of an inhibitor for *Neisseria gonorrhoeae*, J. Bact., 1946, *52*, 453.

(5) Pollock, M. R.: The growth of *H. Pertussis* on media without blood, Brit. J. Exper. Path., 1947, *28*, 295.

(6) Powell, H. M., Culbertson, C. G., and Eminger, P. W.: Charcoal agar culture medium for preparing *Hemophilus pertussis* vaccine, Pub. Health Rep., 1951, *66*, 346.

(7) Stonebrink, F.: Ontwikkeling van een voedingsbodem voor de primaire cultuur van *Mycobacterium tuberculosis* var. *bovis*, Tijdschr. v. diergeneesk., 1952, *77*, 137.

(8) Youmans, G. P., and Karlson, A. G.: Streptomycin sensitivity of tubercle bacilli; studies on recently isolated tubercle bacilli and the development of resistance to streptomycin *in vivo*, Am. Rev. Tubere., 1947, *55*, 529.

(9) Youmans, G. P., and Youmans, A. S.: A method for the determination of the rate of growth of tubercle bacilli by the use of small inocula, J. Bact., 1949, *58*, 247.

(10) Litsky, W., Mallmann, W. L., and Fifield, C. W.: Ethyl violet: A selective dye for the isolation of Gram-negative bacteria, Stain Technol., 1952, *27*, 229.

(11) Whalen, J. W., and Mallmann, W. L.: A comparative study of ethyl violet-azide, Dubos-Davis, and egg media in the primary isolation of tubercle bacilli, paper presented at Michigan Branch S. A. B. Meeting, April, 1952.

(12) Hassler, J. W.: Active Carbon the Modern Purifier, ed. 4, Industrial Chemical Sales Division, West Virginia Pulp and Paper Co., New York, 1941, p. 147.

OBSERVATIONS ON THE PATHOGENICITY OF ISONIAZID-RESISTANT MUTANTS OF TUBERCLE BACILLI FOR TUBERCULOUS PATIENTS[1]

R. OESTREICHER, S. H. DRESSLER, W. F. RUSSELL, JR., J. B. GROW, AND G. MIDDLEBROOK

(Received for publication November 5, 1954)

INTRODUCTION

It is well established that isoniazid-resistant mutants of tubercle bacilli have diminished pathogenicity for guinea pigs (1–6). This attenuation of pathogenicity has been directly related to the physiologic deficiency of such mutants in endogenous catalase activity (5). It manifests itself as an inability of these organisms to multiply in the non-necrotic tissues of the tuberculous-allergic animal.

There is a high incidence of isoniazid-resistant mutants which can be isolated *in vitro* (7) from isoniazid-susceptible populations of tubercle bacilli as well as from many tuberculous patients treated with this agent (8). In spite of this fact, the first report (9) on the clinical results of isoniazid therapy in miliary tuberculosis clearly indicated that, in this disease, "relapse" under treatment with isoniazid (alone) was very rare. Subsequent experience has confirmed this observation. It stands in marked contrast to the previous experience of the same investigators (as confirmed by many others) that relapse under similar conditions (10) in patients on treatment with streptomycin (alone) was not infrequent and was attributable, in most cases, to the failure of streptomycin to inhibit the multiplication of pathogenic, streptomycin-resistant mutants *in vivo* (11).

The analogous situation with regard to pulmonary tuberculosis, on the other hand, has not been so clear. Nearly all investigators have reported "deterioration," "relapse," or "roentgenographic worsening" in isoniazid-treated patients with this form of the disease. In many instances they have related such "unfavorable changes" to the presence of isoniazid-resistant mutants of tubercle bacilli in the sputum. Therefore, it has been assumed by several observers that isoniazid-resistant mutants of tubercle bacilli are, in human beings *as in mice*, either fully pathogenic or insignificantly less pathogenic than isoniazid-susceptible strains.

There is no doubt that episodes of roentgenographic worsening do occur during the course of isoniazid therapy of pulmonary tuberculosis in man. Nevertheless, as pointed out elsewhere (12, 13), whether or not such episodes may justifiably be considered *bona fide progressive* "spreads"[2] seems either to have been ignored or to have been deemed superfluous. Some observers have been im-

[1] From the National Jewish Hospital at Denver, Colorado; and the University of Colorado School of Medicine.

[2] In this context, the term "spread" is used in a generally accepted sense, as defined by Pagel (14): "The original focus has continued its activity and from this focus bronchogenic aspiration has resulted, and new foci appear in new areas of the same or opposite lung."

pressed by the continued general clinical improvement of many patients long after the appearance of isoniazid-resistant mutants in the sputum. In contrast, other observers have been equally impressed by the prompt interruption of such improvement in certain patients at the very time when isoniazid-resistant mutants first rapidly increased in numbers in the sputum. Even this interruption of improvement, without clinical "deterioration," has been interpreted by some investigators as evidence that isoniazid-resistant mutants are not attenuated for man.

It has been emphasized (1) that isoniazid-resistant mutants can multiply freely in previously established, open pulmonary cavities. Hence, it seemed reasonable to the writers to assume that the resumption and continuation of multiplication of drug-resistant tubercle bacilli, in spite of a pronounced decrease in their pathogenicity, could account for the return of mild clinical toxicity. Moreover, the same phenomenon might account for unquestionable roentgenographic manifestations of changes in cavity size and of mechanical disturbances in function, with changes in density in contiguous tissues. (With respect to cavity size, the writers would quote McDermott and co-workers (9) in this connection: "Changes in cavity size of a considerable degree may also occur without representing any real changes in the drug-parasite-host relationships.")

For these reasons, it was deemed necessary to examine other phenomena than these in order to derive from the available clinical material an approximate answer to the question of pathogenicity of isoniazid-resistant tubercle bacilli for human beings—in particular, for those who are already tuberculous.

Progressive, bronchogenic (tubular) "spread" is an integral step in the natural history of progressive open, cavitary, pulmonary tuberculosis; its frequency is high in untreated, advanced disease, when the sputum is persistently positive for tubercle bacilli. It is generally accepted that, in patients with this type of disease who have been treated with streptomycin and who persistently excrete streptomycin-resistant bacilli, progressive "spreads" do not necessarily occur, but they are not uncommon, regardless of whether or not streptomycin is administered (15).

The mechanics of progressive, bronchogenic spread are understood. Living tubercle bacilli, discharged into the draining bronchus, are aspirated into previously uninvolved parenchyma, there to initiate and continue multiplication and to produce lesions of tuberculous pneumonia, necrosis and, frequently, excavation.

Experimental observations in guinea pigs have revealed that isoniazid-resistant mutants of tubercle bacilli are strictly limited in their ability, once they have been implanted, to continue multiplication in previously uninvolved parenchyma of guinea pig lungs (5).

It seemed plausible, therefore, to conclude that any attenuation of isoniazid-resistant mutants for human beings should manifest itself *primarily in a significantly decreased incidence of progressive bronchogenic spreads of tuberculous infection in patients with pulmonary tuberculosis who are under treatment with*

isoniazid. That the incidence of such "spreads" may well be diminished under these conditions has been indicated by the Cornell investigators (12), who have employed the phrase "prolongation of total antimicrobial action" to describe this phenomenon.

It has already been pointed out that apparent "relapses" do occur in isoniazid-treated patients. Mechanical atelectases and the well-known, though not so well-defined, clinical entity, "epituberculosis," may fairly be differentiated from *bona fide* bronchogenic "spread" in any patient with pulmonary tuberculosis only by the subsequent course of events. In the former conditions, the roentgenographic densities and the occasionally accompanying evidences of clinical toxicity appear with some suddenness, promptly reach maximum intensity, and disappear either slowly or rapidly. On the other hand, the *progressive* character of bronchogenic spread of tuberculous infection in new areas of the lungs, in most cases, clearly distinguishes it from the former entities. There is no reason, in the opinion of the writers, to believe that those conditions which lead to manifestations of atelectasis or "epituberculosis" might be affected by isoniazid therapy, except possibly to be increased.[3]

The purpose of this paper is to present the results of the writers' practice of maintaining previously treated, persistently "sputum-positive" patients on long-term, ambulatory treatment with isoniazid alone. This practice was followed without changing regimen—without adding other antituberculous agents and, in most cases, without discontinuing isoniazid—in the face of clinical, roentgenographic, or other change which might have been hastily interpreted as "spread" caused by fully pathogenic tubercle bacilli.

MATERIALS AND METHODS

The cases selected for this study were derived from the adult medical service and the follow-up department of the National Jewish Hospital during the past two and a half years. The prolonged administration of isoniazid to all patients in the study had been preceded by treatment with either streptomycin, para-aminosalicylic acid (PAS), or viomycin, or combinations of these agents. This pre-isoniazid treatment had been given prior to admission to the hospital or previously during the present hospitalization. Thus, the cases followed for this study are obviously derived from a much larger group of patients whose sputum in most instances became negative for tubercle bacilli or was not persistently positive after treatment with isoniazid in combination with other agents.

Accordingly, all tuberculous patients with advanced pulmonary tuberculosis whose course strictly satisfied the following criteria were included in the study:

1. *Persistently positive sputum specimens* (both smear and culture) containing large numbers of mutants of typical, mammalian-type tubercle bacilli resistant to at least

[3] There is a possibility that, under certain ill-defined conditions, isoniazid-resistant mutants which are catalase-negative and highly attenuated for guinea pigs may, nevertheless, be capable of proliferating in tuberculous lymph nodes. This is supported by some observations of other investigators (9, 12) and by observations in a few isoniazid-treated children, and in at least one adult with mediastinal tuberculous nodes who is at the National Jewish Hospital. These observations will be described elsewhere.

1γ of isoniazid per ml. of medium during an observation period of not less than six months.

2. *Treatment with isoniazid alone* in daily dosage of 4 to 16 mg. per kg.[4] during the entire period of observation, with the following exceptions:

In 4 patients (one permanently; 3 for only one to three and one-half months) isoniazid was discontinued because of drug toxicity or idiosyncrasy, but no other antituberculous agent was employed.

In 10 patients isoniazid was discontinued for one month, in order to afford a period of therapeutic trial of another agent. The trial agent had, during this period, no qualitatively or quantitatively detected clinical or bacteriologic influence whatsoever on the tuberculous processes in these patients; isoniazid was continued thereafter in the same dosage without further interruption.

Eight of the patients included in this study received streptomycin for short periods of time during the observation period. These patients, nevertheless, were included in the study because *large numbers* of streptomycin-resistant tubercle bacilli were repeatedly isolated from their sputum before, during, and after these periods of administration of streptomycin.

The bacteriologic methods applied in this study have been described (16). They consisted of concentration, examination of stained smears, decontamination, culture, drug-susceptibility studies by the direct method, and catalase tests on each specimen. Specimens were usually examined at monthly intervals, or more frequently.

Standard postero-anterior roentgenograms of the chest were taken at intervals of four to six weeks, or more frequently. They were routinely examined by both the roentgenologist and the entire medical and surgical house staff. Lateral views, apical lordotic, oblique, and tomographic studies were done, whenever indicated.

Nearly all patients in this study received thiamine (100 mg. per day by mouth) and pyridoxine (25 to 100 mg. per day by mouth) during the period of observation. In general, no other nutritional supplements were prescribed.

If there was cause to suspect that a patient was not taking isoniazid by mouth, it was administered by the intramuscular route.

All patients in this study were permitted physical activity as described in a previous communication (17). In the absence of clinical evidence of toxicity, strict bed rest was never prescribed. However, 4 of the patients included in this study suffered such limitation of cardiopulmonary function that they were unable to enjoy much physical activity.

Forty-five patients are available for this report, 21 females and 24 males. The average age of the females was thirty-six years; of the males, forty-three years.

Clinical or roentgenographic evidence of *progressive* tuberculous disease had been present in all but 13 of the 45 patients of this study within less than twelve months before initiation of chemotherapy with isoniazid. The tuberculous processes in these 13 patients had been "active" but "stabilized" with streptomycin,

[4] In general, all patients in this study who were treated with isoniazid at this hospital after April, 1953, received this drug in daily dosages of 8 mg. per kg. in two divided doses. Thus, each patient was maintained, for the most part, on this dosage for his respective observation period.

PAS, or viomycin, alone or with various combinations of these agents, either intermittently or continuously, for periods longer than twelve months before treatment with isoniazid was started.

OBSERVATIONS

The 45 patients of this study have thus far been observed, under conditions previously described, for the following periods of time: 11 for six to eight months; 17 for nine to twelve months; 12 for thirteen to eighteen months; and 5 for eighteen to twenty-five months. Thus, there was an average of twelve months' observation time per patient.

Roentgenographic Course During the Prolonged Isoniazid Therapy of Patients with "Isoniazid-Resistant" Tubercle Bacilli

No significant roentgenographic change

In 32 patients there were no significant changes in serial chest roentgenograms except for changes obviously caused by surgical procedures.

Roentgenographic improvement

Two patients showed marked improvement. One of the 2 had far advanced silicotuberculosis with large cavities on both sides. The roentgenographic shadows regressed and cavities strikingly decreased in size in the comparatively short period of ten months. The patient was recently discharged home and has been leading a relatively active life. The second case was that of an elderly white man who had a large cavity in the apical region and had developed a fresh, smaller cavity in the lower field of the same lung while on a low-dosage, probably irregular, regimen of isoniazid therapy. On admission the sputum was highly positive for *Mycobacterium tuberculosis*, and the strain was found to be resistant to both streptomycin and isoniazid and was catalase-positive. At that point, isoniazid dosage was increased to 8 mg. per kg. of body weight per day. Within three months the "fresh" cavity disappeared and was not demonstrable even on tomograms. During the same period extensive flocculant shadows in the contralateral lung had greatly cleared.

"Roentgenographic worsening"

Eleven patients showed "roentgenographic worsening" which might, on short-term evaluation, have been interpreted as evidence of "deterioration" or "relapse." These changes can be roughly divided into three categories: increases in cavity size, "transient infiltrates," and new, more persistent shadows.

Increase in cavity size: There were 4 patients whose cavities became progressively larger and the cavity walls thinner.[5] The clinical course of one of these patients (D-L.T.) is described below.

[5] Although the ultimate fate of such cavities may, in some cases, be sterilization of the cavity lining, the ever-increasing size may seriously embarrass respiratory function. Increase of size of such cavities is usually caused by coughing, with consequent expansion of the thin cavity wall, which is devoid of elastic tissue and cannot, therefore, return to its former state.

"Transient infiltrates": In 4 patients there were "transient infiltrates." One patient developed, after classical thoracoplasty, an extensive infiltration in the contralateral lower lung field, for all practical purposes indistinguishable from a tuberculous bronchopneumonic lesion. Under continuous isoniazid therapy, this lesion cleared within a few weeks.

Another patient developed, without any clinical symptoms or signs, multiple infiltrations in previously affected areas. The only abnormal finding at that time was an increased erythrocyte sedimentation rate. The most likely explanation for this event was spilling of softened caseous cavitary contents into the bronchial tree, aspiration, and subsequent bronchial embolism. The "lesions," if any, caused by the attenuated microorganisms were short-lived, and within a few weeks the newly developed shadows regressed completely.

The third patient with "transient infiltrate," a 39-year-old white, married female, was admitted to the hospital with far advanced, but mainly unilateral cavitary, disease. Isoniazid treatment was started in April, 1952. In May, 1952, isoniazid-resistant tubercle bacilli were isolated from her sputum for the first time. Isoniazid resistance persisted; the bacilli were catalase-negative throughout her stay. A seven-rib thoracoplasty was planned in the summer of 1953. However, in July, 1953, a routine roentgenogram revealed a very marked increase of density between the lateral wall of the cavity in the right mid-field and the chest wall. At the same time her temperature rose to 100°F. and her erythrocyte sedimentation rate, which was 13 mm. in May, rose to 51 mm. The patient was febrile for only a few days, and on subsequent roentgenograms the new shadows regressed and had disappeared completely by October, 1953, when thoracoplasty was eventually performed. Subsequently, in September, 1954, the patient had a resection of the right upper lobe; her sputum, however, remained positive, with isoniazid-resistant tubercle bacilli.

The fourth case was that of a patient with a completely destroyed left lung who showed slight increase of a previously present contralateral shadow. At the same time temperature elevation, anorexia, and malaise were observed for a few days only. Within four weeks this shadow returned to its former size and appearance.

New persistent densities: In 2 of the 45 patients, new "lesions" suddenly appeared and persisted during the observation period. These shadows showed only as much regression as some previously untreated tuberculous lesions show after antimicrobial treatment has first been initiated. The first of these two new lesions developed immediately after extensive unilateral "classical" thoracoplasty. The lesion appeared in the lower contralateral lung field and regressed slowly during a period of seventeen months' observation (clinical course of this patient (C. R.) is described below).

The other instance of a new lesion occurred in a 48-year-old white man (D-V. C.) who had far advanced tuberculosis of the right lung and diffuse dilatation of the aorta of undetermined origin. He was admitted in November, 1953, and, as he had received isoniazid therapy before his admission, his microorganisms were found to be isoniazid-resistant and catalase-negative. For diagnostic reasons, angiocardiography was performed on January 30, 1954. For

technical reasons, only the pulmonary vessels were roentgenographically demonstrated. There was no immediate untoward reaction. However, from February 7 to February 21, 1954, he had slight fever (99° to 100°F.), with no other symptoms or signs. A roentgenogram, taken on March 12, 1954, showed new, diffuse, mottled shadows in a hitherto apparently unaffected area in the left mid-lung field (probably lingula). The erythrocyte sedimentation rate showed only a slight rise.

Under continued isoniazid therapy the patient remained asymptomatic and the roentgenographic shadows regressed slowly over a period of four months. At the end of this time, however, a second sudden rise of temperature to 103.4°F. occurred on June 29, 1954. Penicillin (300,000 units twice daily, intramuscularly) and erythromycin (0.4 gm. three times daily by mouth) were administered. The fever disappeared in twenty-four hours and body temperature remained normal thereafter (more than four months). A roentgenogram of the chest on July 6, 1954, revealed new shadows in an area adjacent to the previously noted shadows in the lingula of the left upper lobe. These new shadows, in turn, regressed markedly over the next four months. Tomograms on October 5, 1954, showed a small area of rarefaction in the lingula.

The peculiar circumstances under which these two "relapses" occurred in this patient merit consideration. There was marked anatomic distortion of his thoracic viscera, caused by the enormous enlargement of the aorta (12 cm. in diameter at the third rib anteriorly in ordinary postero-anterior chest roentgenogram). The sudden appearance and remissions of clinical illness, which were apparently associated with the development of new shadows in the lingula, were not typical of progressive tuberculous infection.

It has been concluded that no definite opinion is justified of the possible role of multiplication of isoniazid-resistant, catalase-negative tubercle bacilli in the etiology of these new lesions in this patient.

A third patient (mentioned below under *Clinical Course*) developed an interlobar effusion during the period of observation, with localized pain and pleural rub, but without rise of temperature or of erythrocyte sedimentation rate.

Results of Bacterial Catalase Tests

Catalase tests (16) were performed on several cultures from each patient during the course of this study (beginning in January, 1954). The results may be summarized as follows: 25 patients yielded only catalase-negative, resistant organisms; 17 patients yielded catalase-positive, resistant organisms—rarely in pure culture—most commonly in variable proportions with catalase-negative, resistant bacilli; and the organisms from one patient were not tested for catalase activity.

The remaining 2 patients yielded only catalase-negative, isoniazid-resistant cultures during the earlier period of study and "special mixed" cultures with some catalase-positive, resistant bacilli later. These 2 patients, whose isoniazid therapy had been started at no less than 8 mg. per kg. per day, were subsequently treated with only half this dosage. There was a variable period of time

after the reduction in dosage before the catalase-positive, resistant mutants first made their appearance. Subsequent increase in dosage did not effect disappearance of the catalase-positive members of the population from their sputum.

Clinical Course

Body weight: Nearly all of the patients in the study either gained weight slowly or maintained their previous weights. Only changes of plus or minus 10 per cent or more were deemed, in this study, to be significant for evaluation. The chosen "base line" was the weight of the patient at the beginning of the observation period.

Thus, 4 patients gained 10 per cent or more in weight; 9 patients (2 of whom were obese) lost 10 per cent or more of their weight; and there were variations of less than plus or minus 10 per cent in 32 patients.

The following patients suffered significant weight losses:

C. R. (catalase-positive, isoniazid-resistant bacilli): A weight loss of 21 per cent occurred in this patient within the first nine months of the observation period of 22 months. During this period the patient had an extraperiosteal plastic sponge plombage; the sponge had to be removed later because of unspecific, probably nonmicrobial suppuration (allergic to sponge material) of the extraperiosteal space, and a "classical" thoracoplasty was performed. At that time isoniazid therapy had to be discontinued because of severe neurotoxic symptoms (*see* case No. 6 in reference 18). Repeated attempts to reinstitute isoniazid therapy along with pyridoxine were unsuccessful. Since the discontinuation of isoniazid therapy, however, during the last thirteen months of the observation period, this patient regained 24 of the 30 pounds previously lost. His neurotoxic symptoms persisted, unchanged.

D-L. T. (catalase-negative, isoniazid-resistant bacilli): A 12 per cent weight loss occurred during the period of eleven months' observation in this patient whose cavitary lesion showed bizarre changes in the sense of increase of cavitary diameter as seen on postero-anterior and tomographic films, with concomitant thinning out of the cavitary wall. This appearance changed several times to thickening of cavitary wall, decrease of the cavitary diameter, and increase of pericavitary shadows. This was attributed to a valvular mechanism in the draining bronchus of the cavity. During these spells the patient coughed a great deal, his appetite was poor, and weight loss occurred.

D. M. (catalase-negative, isoniazid-resistant bacilli): This patient experienced a weight loss of 12 per cent during a period of nineteen months. A "classical" thoracoplasty and subsequent resection of the right upper lobe were performed within the observation period. The last operation was performed only two months before the end of the observation period. The episodes of weight loss were specifically associated with the operative procedures.

L. R. W. (catalase-negative, isoniazid-resistant bacilli): This patient had a 12 per cent weight loss which was attributed to persistent drainage from a bronchopleurocutaneous fistula which was resected only six weeks before the end of the observation period of twenty-five months.

H. D. M. (catalase-positive, isoniazid-resistant bacilli): A 10 per cent weight loss during the first nine months of the eighteen months' observation occurred in this patient. She had had extensive thoracoplasty before admission to this hospital and was left with gross cavitation on both sides. During her hospital stay, this patient had, in her pre-menstrual periods, a severe increase in coughing and, concomitant with that, bouts of vomiting. Consequently there was rather marked fluctuation of weight associated with such episodes (weight in the last week of March, 1953, 99 pounds; in the

first week of April, 1953, 94.75 pounds). Similar episodes occurred at monthly or bimonthly intervals. The weight loss in this patient was, therefore, attributed to nontuberculous causes.

K. M. (catalase-positive, isoniazid-resistant bacilli): A 23 per cent weight loss occurred in this patient. She was a 34-year-old white woman who had had extensive streptomycin-PAS therapy in other institutions as well as isoniazid therapy which, too, was started before admission to this hospital. During the two years immediately prior to her admission, her condition was more or less stationary with little change in weight. Her admission weight was 93 pounds and was stationary until August 11, 1953, when an extraperiosteal plombage was performed on the left side. Three weeks later her weight was 82 pounds. After the operation the patient went into acute heart failure, with edema of the legs, arms, and even of the face. On August 24, 1953, her total serum proteins were only 5.3 gm. per 100 ml. with an inverted albumin-globulin ratio. During the next five months her condition was precarious. By October 12, 1953, she had lost another 8 pounds; this was attributable, however, to digitalization and diuresis. By January 10, 1954, her condition had improved to such an extent—her weight rose to 79 pounds and no edema was present—that an open cavernostomy was performed and continuous suction drainage (Monaldi) was started. Her condition subsequently improved but very little; her sputum remained highly positive for tubercle bacilli and her weight was 74 pounds at the end of the observation period of twenty months.

The failure of this patient to maintain or gain weight was attributed to both her cardiopulmonary insufficiency and continuous drainage of large amounts of protein from an open cavernostomy.

T. J. (catalase-positive, isoniazid-resistant bacilli): A 20 per cent weight loss occurred in this patient who had moderate to severe hemoptyses during the observation period (ten months). One month after the beginning of the observation period, an extraperiosteal sponge plombage was performed without influence on the hemoptyses. One month before the end of the observation period a pneumonectomy was performed; however, the patient's sputum remained positive for tubercle bacilli because of previously present contralateral cavitation.

Two additional patients lost more than 10 per cent of their weight intentionally, because of obesity.

Erythrocyte sedimentation rates: With regard to erythrocyte sedimentation rates, there was no relationship between anatomic extents of disease and actual sedimentation rates. It was noted that even among those patients who had normal sedimentation rates there were some who had recently had pleural effusions or severe cough and expectoration with probable secondary bronchopulmonary infections. On the other hand, one patient has been well and performing a full day's work, five days a week, for the last year and has had no "toxic" manifestations whatsoever, in spite of a consistently elevated erythrocyte sedimentation rate during the entire observation period.

Symptoms or signs of toxicity: There were several patients, however, who manifested clinical symptoms or signs of "toxicity" which could be explained only on the basis of persistent multiplication of tubercle bacilli in open cavities, with absorption of the metabolic products of these organisms.

Eight patients (6 females and 2 males) experienced brief episodes of temperature elevation up to 101°F. by mouth, associated with increased cough, expectoration and, in some instances, chest pain and wheezing. Antimicrobial therapy with penicillin, erythromycin, or one of the tetracyclines often failed to lower

the body temperature to normal. Among these patients was D-L. T. (described above) who had fever regularly associated with the already mentioned roentgenographic changes.

One patient (H. D. M.) had severe coughing spells (referred to above under *Body weight*), associated with vomiting, usually at the beginning of her menstrual period. It is interesting to note that these episodes continued unabated even after reversal of infectiousness, which ultimately occurred (before sectional surgery) after twenty months of excreting isoniazid-resistant tubercle bacilli. This patient had extensive bronchiectatic changes in both upper lobes, and it is possible that the edema which is seen in some women in the pre-menstruum may have caused partial bronchial obstruction. The patient has subsequently had a resection of residual cavitary lesions and her general condition has improved.

Four patients were severely debilitated by cardiopulmonary insufficiency. Three of these patients had had extensive collapse or sectional therapy (2 had seven-rib thoracoplasties, and one had an extraperiosteal sponge plombage and open cavernostomy drainage), and the fourth patient had cor pulmonale on admission to the hospital. Their limitations of pulmonary function markedly restricted their physical activities.

One patient mentioned previously developed a small interlobar effusion during the observation period. There was, however, no elevation of temperature or erythrocyte sedimentation rate at this time.

Six patients had slight to moderate pulmonary hemorrhages. Three of these had had hemoptyses before the observation period, but the other 3 had pulmonary hemorrhages (one mild, 2 moderately severe) for the first time during the observation period.

Surgical Procedures

Twenty major pulmonary surgical procedures were performed on 15 of the 45 patients during the observation period. There were twelve extraperiosteal sponge plombages; two "classical" thoracoplasties; five sectional procedures (lobectomies or pneumonectomies); and one cavernostomy with open drainage (Monaldi).

In all but 5 patients, these operations were performed as preliminary procedures to additional, more drastic interventions, with the ultimate goal to rid the patient of the source of his bacilliferous sputum. Two operations (a pneumonectomy for persistent hemorrhage, and a lobectomy, with excision of a bronchopleurocutaneous fistula) were performed for obviously urgent indications.

It is important to note that the above-described surgical procedures failed to cause significant decreases in the numbers of isoniazid-resistant tubercle bacilli in the sputum of these patients; otherwise, they would not have been included in this study.

DISCUSSION

It has already been pointed out (12, 13) that the *nature* of "roentgenographic worsening" which has been reported to occur in some tuberculous patients dur-

ing therapy with isoniazid has received little attention. Details of such episodes have not been catalogued in any of the large-scale cooperative investigations on the chemotherapy of tuberculosis.

The "relapses" (with possibly one exception, the case of D-V. C) observed in the present study should not, in the writers' opinion, be considered bona fide, progressive, bronchogenic spreads of tuberculous infection. Failure of clinical or roentgenographic progression, despite continuance of the isoniazid alone and no prolonged bed-rest treatment or other intervention, makes it probable that any tubercle bacilli aspirated into the previously uninvolved lung of these patients were incapable of setting up progressive tuberculous processes by multiplication. Had these cases, and some 6 others[6] which have been encountered at the National Jewish Hospital, been hastily assumed to represent "true spread," their therapy might have been changed. Regression of clinical signs and symptoms and of roentgenographic shadows, or their failure to progress, might then have been attributable to the change in therapeutic regimen.

It is also noteworthy that none of the other untoward events have occurred in patients on isoniazid therapy at the National Jewish Hospital, such as the development of tuberculous laryngitis, enteritis, miliary disease or meningitis, which were not uncommonly observed to develop in such persistently infectious, untreated cases during similar observation periods before the chemotherapeutic era.

In the writers' opinion, the most important single event in the microbe-drug-host relationship which is responsible for the "prolongation of total antimicrobial activity" (12) of isoniazid in tuberculous patients is microbial mutation to deficiency in endogenous catalase activity and deficiency in pathogenicity simultaneously with mutation to isoniazid-resistance.

In general, therefore, it may be suggested that isoniazid *in adequate dosage* (16) achieves "physiologic imprisonment" (12) of tubercle bacilli in a threefold fashion in man:

> It inhibits the multiplication of drug-susceptible organisms and, probably, under certain circumstances sterilizes them.
>
> It loses this direct antimicrobial activity only when the parasite suffers genetic loss of a physiologic function which is essential for its full pathogenicity.
>
> Its continued administration not only attacks the natively susceptible parasite which may be given an opportunity to multiply after long periods of metabolic quiescence in necrotic lesions; it also attacks any drug-susceptible, reverse-mutants which may make their appearance from drug-resistant populations.

Perhaps it is only a slight distortion of the facts to conclude that isoniazid is a remarkably unique chemotherapeutic agent. It attacks the susceptible parasite, while the host starves the drug-resistant mutant.

* * *

Many aspects of isoniazid therapy of tuberculosis remain to be explored. Not the least important of these may be the possible difference in clinical significance

[6] Excluded from this report because of failure to satisfy all of the criteria specified for selection for this study.

of the two principal types of isoniazid-resistant mutants of tubercle bacilli which have been described: catalase-negative, which are highly attenuated for guinea pigs; and catalase-positive, which manifest various degrees of deficiency in this enzyme activity and roughly comparable degrees of attenuation for guinea pigs, from slight to moderate. Either type, or both, have been found in the sputum of isoniazid-treated tuberculous patients. However, as previously pointed out (16), the higher doses of isoniazid (no less than 8 mg. per kg. per day) usually prevent the emergence of the catalase-positive resistant mutants *in vivo*.

The catalase-positive, isoniazid-resistant mutants often manifest so little attenuation for the guinea pig that they may, without strictly quantitative investigation, appear to be fully pathogenic for these animals. Therefore, it has been anticipated that such strains may well be potential agents of progressive "spreads" of tuberculous disease in man, both endogenous and exogenous (from the treated patient to other human beings). On the other hand, it is possible that a slight degree of attenuation of tubercle bacilli may manifest itself in a highly significant manner, clinically, in the partially immune tuberculous patient. In any event, the writers have failed to recognize any sign that the 17 patients in this study who excreted large numbers of isoniazid-resistant, catalase-positive bacilli fared less well than those 25 patients who excreted only catalase-negative organisms throughout the period of observation.

Adequate study of this problem will obviously require observation and investigation of larger numbers of patients than the present writers have had at their disposal.

Another facet of the pathogenicity of catalase-deficient tubercle bacilli for human beings is the obvious complexity of the problem as it may apply to a particular individual. If the many variables of the growth environment which have already been seen (5) to determine whether or not catalase-negative strains will multiply *in vitro* are actually operative *in vivo*, then it is probable that the nutritional state of the specific host, as well as its ability to respond with an adequate inflammatory reaction, will play an important role in this parasite-host relationship. Thus, for example, the manganese content of the diet (5) and the possible existence of a nutritional and consequent host-tissue deficiency of this metal may be decisive in determining an unfavorable outcome of an infection with catalase-negative strains of tubercle bacilli in some patients.

Needless to say, there are probably many other, as yet unknown, variables which could tip the equilibrium in one direction or another. Suffice it to predict, therefore, that unequivocally progressive tuberculous infection and disease will be encountered in some persons as a result of multiplication of catalase-negative strains of isoniazid-resistant tubercle bacilli. To emphasize this probability, one may refer to the peculiar susceptibility of some rare human beings to strains of tubercle bacilli (BCG) which are deficient in pathogenicity for the vast majority of human beings (19, 20).[7]

[7] The writers do not wish to imply by this reference the actual order of magnitude of human susceptibility to catalase-deficient strains of tubercle bacilli. If an opinion may be expressed, susceptibility to such strains should be much more common than to BCG.

Lest there be some misunderstanding of the implications of the present experiences indicating a strictly limited pathogenicity of most isoniazid-resistant mutants for most tuberculous patients, the writers would like to express a firm conviction; namely, a "dead" tubercle bacillus will probably always be preferred to a living tubercle bacillus, even though the latter, because of its deficiency in catalase activity, may be highly attenuated for the great majority of human beings.

Acceptance of the evidence that isoniazid-resistant, catalase-deficient strains of tubercle bacilli have altered pathogenic potentialities for human beings entails certain revisions in views and practices of chemotherapy in tuberculosis. Nevertheless, in the writers' opinion, no efforts should be spared to improve on presently available means of "enhancing" the antimicrobial action of antituberculous agents during the early phase of therapy. All efforts should be continued toward finding means to render as many patients as possible bacteriologically negative as soon as possible after initiation of chemotherapy.

To state our views in other words: If continuing multiplication of tubercle bacilli *in vivo* cannot be avoided, let such organisms, at the very least, be highly resistant to the direct antibacterial effects of isoniazid, i.e. "catalase-negative." Furthermore, let their presence not interfere with appropriate surgical efforts to close or remove the cavities in which these organisms multiply.

The evidences of mild toxicity observed in several of the patients in this study are interpreted as the inevitable consequence of extensive multiplication of the attenuated tubercle bacilli in open cavities. These parasites appear to be indistinguishable from the typical, fully virulent isoniazid-susceptible parent organisms from which they are derived, except for their requirement of a ready external source of soluble catalase for multiplication. Provided with exogenous catalase, they form "serpentine cords"; they produce "tuberculin" on autolysis; they elicit the classical Koch phenomenon in tuberculin-hypersensitive animals. Thus, their "poisonous" characteristics[8] have not been found to differ from those of their fully pathogenic, catalase-positive, isoniazid-susceptible parent organisms. Their "attenuation" appears to manifest itself exclusively in loss of ability to multiply progressively in new areas of parenchymal tissue in the hypersensitive host.

Continued proliferation of attenuated, isoniazid-resistant bacilli in the cavity, their excretion into the draining bronchus, and the accompanying inflammatory responses in the allergic host result in a state of equilibrium between host and parasite which is not consistent with "good health" in many persons. Furthermore, the pathologic processes which almost invariably follow on the prolonged periods of coughing suffered by many such patients may be anticipated. It may

[8] Because of etymologic considerations and their possible implications, the term "virulence" has been purposely avoided in this report, notwithstanding previous definitions (21, 22) of the word. The isoniazid-resistant, catalase-deficient mutants, although they manifest important attenuation in pathogenicity, might not be considered attenuated in "virulence": the *poisonous qualities* of their metabolic products may be viewed as essential but not sufficient for full pathogenicity.

even be suggested that repeated aspiration of these "functionally sterile" organisms and of purulent cavitary secretions during prolonged periods into normal pulmonary parenchyma could ultimately induce pathologic changes resulting in seriously progressive limitation of cardiopulmonary function.

SUMMARY

Observations are described on 45 patients with advanced pulmonary tuberculosis who persistently excreted isoniazid-resistant mutants of tubercle bacilli but were maintained on chemotherapy with isoniazid alone for periods of six to twenty-five months (average of one year per patient) after completing preliminary combined drug therapy.

Failure of new lesions of progressive tuberculous disease to develop in any, except possibly one, of these patients under these conditions is consistent with certain observations of other investigators on the treatment of tuberculosis with this agent.

These experiences are interpreted as indirect evidence that the pathogenic potentialities of most isoniazid-resistant mutants of typical mammalian-type tubercle bacilli are strictly limited in an important manner for most tuberculous patients.

SUMARIO

Observaciones Relativas a la Patogenicidad de las Mutantes Isoniacidorresistentes de Bacilos Tuberculosos para los Tuberculosos

Describense las observaciones realizadas en 45 enfermos que tenían tuberculosis pulmonar avanzada y excretaban persistentemente mutantes isoniacidorresistentes de bacilos tuberculosos, pero que fueron mantenidos a quimioterapia con la isoniacida sola durante perfodos de seis a veinticinco meses (promediando un año por persona) después de terminar la farmacoterapia combinada preliminar.

La falta de aparición de nuevas lesiones de afección tuberculosa evolutiva en todos estos sujetos, con la posible excepción de uno, en dichas condiciones, es compatible con ciertas observaciones de otros investigadores acerca del tratamiento de la tuberculosis con dicho agente.

Interprétanse estas observaciones como prueba indirecta de que las potencialidades patógenas de la mayor parte de las mutantes isoniacidorresistentes de los bacilos tuberculosos de típico tipo mamífero están rígidamente limitadas en forma importante para la mayor parte de los tuberculosos.

RESUME

Observations sur la pathogénicité des mutants isoniazide-résistants du bacille de Koch chez le malade tuberculeux

Description des observations effectuées chez 45 malades atteints de tuberculose avancée avec excrétion persistante de mutants isoniazide-résistants de bacilles tuberculeux, mais qui étaient maintenus à la chimiothérapie par l'isoniazide seule, pendant des périodes de six à vingt-cinq mois (en moyenne un an pour chaque malade) après achèvement de la cure préliminaire par association médicamenteuse.

Dans ces conditions, le défaut d'apparition de nouvelles lésions tuberculeuses évolutives dans tous ces cas, à l'exception possible d'un seul, corrobore certaines observations d'autres chercheurs sur le traitement de la tuberculose par l'isoniazide.

Ces observations sont interprétées comme fournissant la preuve indirecte que, chez la plupart des tuberculeux, il existe une stricte limitation du pouvoir pathogène de la plupart de mutants isoniazide-résistants du bacille tuberculeux, type mammifère.

REFERENCES

(1) MIDDLEBROOK, G., AND COHN, M. L.: Some observations on the pathogenicity of isoniazid-resistant variants of tubercle bacilli, Science, 1953, *118*, 297.

(2) MITCHISON, D. A.: Tubercle bacilli resistant to isoniazid: Virulence and response to treatment in guinea pigs, Brit. M. J., 1954, No. 4854, 128.

(3) MORSE, W. C., WEISER, O. L., KUHNS, D. M., FUSILLO, M., DAIL, M. C., AND EVANS, J. R.: Study of the virulence of isoniazid-resistant tubercle bacilli in guinea pigs and mice, Am. Rev. Tuberc., 1954, *69*, 464.

(4) MEISSNER, G.: Zur Frage der Virulenz chemo-resistenter Tuberkelbakterien: II. Mitt. In vivo INH resistent gewordene Tuberkelbakterien, Beitr. z. Klin. Tuberk., 1954, *110*, 538.

(5) COHN, M. L., KOVITZ, C., ODA, U., AND MIDDLEBROOK, G.: Studies on isoniazid and tubercle bacilli: II. The growth requirements, catalase activities, and pathogenic properties of isoniazid-resistant mutants, Am. Rev. Tuberc., 1954, *70*, 641.

(6) PEIZER, L. R., MINKIN, A., AND WIDELOCK, D.: A further study of virulence in guinea pigs of isoniazid-resistant tubercle bacilli isolated from clinical material, Am. Rev. Tuberc., 1954, *70*, 728.

(7) MIDDLEBROOK, G.: Sterilization of tubercle bacilli by isonicotinic acid hydrazide and the incidence of variants resistant to the drug *in vitro*, Am. Rev. Tuberc., 1952, *65*, 765.

(8) STEENKEN, W., JR., MEADE, G. M., WOLINSKY, E., AND COATES, E. O., JR.: Demonstration of increased drug resistance of tubercle bacilli from patients treated with hydrazines of isonicotinic acid, Am. Rev. Tuberc., 1952, *65*, 754.

(9) CLARK, C. M., ELMENDORF, D. F., JR., CAWTHON, W. U., MUSCHENHEIM, C., AND MCDERMOTT, W.: Isoniazid (isonicotinic acid hydrazide) in the treatment of miliary and meningeal tuberculosis, Am. Rev. Tuberc., 1952, *66*, 391.

(10) MCDERMOTT, W., MUSCHENHEIM, C., HADLEY, S. J., BUNN, P. A., AND GORMAN, R. V.: Streptomycin in the treatment of tuberculosis in humans: I. Meningitis and generalized hematogenous tuberculosis, Ann. Int. Med., 1947, *27*, 769.

(11) MCDERMOTT, W.: Significance of finding of tubercle bacilli resistant to streptomycin *in vitro* in antimicrobial therapy of tuberculosis, Bull. New York Acad. Med., 1948, *24*, 131.

(12) DEUSCHLE, K., ORMOND, L., ELMENDORF, D., JR., MUSCHENHEIM, C., AND MCDERMOTT, W.: The course of pulmonary tuberculosis during long-term single-drug (isoniazid) therapy, Am. Rev. Tuberc., 1954, *70*, 228.

(13) MIDDLEBROOK, G., AND DRESSLER, S. H.: Clinical evaluation of isoniazid, Am. Rev. Tuberc., 1954, *70*, 1102.

(14) Pulmonary Tuberculosis, edited by Pagel, W., Simmonds, F. A. H., MacDonald, N., and Fatti, L., ed. 2. Oxford University Press, London, 1948, p. 338.

(15) MUSCHENHEIM, C., MCDERMOTT, W., HADLEY, S. J., HULL-SMITH, H., AND TRACY, A.: Streptomycin in the treatment of tuberculosis in humans: II. Pulmonary tuberculosis, Ann. Int. Med., 1947, *27*, 989.

(16) MIDDLEBROOK, G., COHN, M. L., AND SCHAEFER, W. B.: Studies on isoniazid and tubercle bacilli: III. The isolation, drug-susceptibility, and catalase-testing of tubercle bacilli from isoniazid-treated patients, Am. Rev. Tuberc., 1954, *70*, 852.

(17) DRESSLER, S. H., ANTHONY, E. M., RUSSELL, W. F., JR., GROW, J. B., DENST, J., COHN, M. L., AND MIDDLEBROOK, G.: Ambulation of patients with pulmonary tuberculosis under protection of chemotherapy, Am. Rev. Tuberc., 1954, *70*, 1030.

(18) Oestreicher, R., Dressler, S. H., and Middlebrook, G.: Peripheral neuritis in tuberculous patients treated with isoniazid, Am. Rev. Tubere., 1954, *70*, 504.

(19) Generalized BCG infection in man, Acta tubere. Scandinav., 1954, *29*.

I. Tharp-Meyer, H.: Clinical report, p. 173.

II. Oeding, P., and Hesselberg, I.: Bacteriological investigations, p. 180.

III. Waaler, E., and Oeding, P.: Autopsy findings, p. 188.

(20) Meyer, J.: A fatal case of tuberculosis produced by BCG, Am. Rev. Tubere., 1954, *70*, 402.

(21) Rich, A. R.: The Pathogenesis of Tuberculosis, Charles C Thomas, Springfield, Illinois, 1944, p. 101.

(22) Middlebrook, G.: The experimental analysis of virulence of tubercle bacilli, Bull. New York Acad. Med., 1950, *26*, 468.

METHODS OF FINDING TUBERCULOSIS CASES AND REACTORS[1, 2]

R. J. ANDERSON

(Received for publication May 18, 1954)

Because of the many great and rapid technical and program advances which we have seen in recent years, we are today reaching new milestones of tuberculosis control so rapidly that it has become difficult to equate our present and future activities with the traditional concepts of public health action.

New drugs—cheap, easily administered, and effective—are helping to change therapy as we have known it. The drugs have made it possible for many patients to receive treatment at home, and many of these, under efficient supervision and care, seem to be doing well.

Before we can say what role home treatment will play in the life of the patient and the community, evidence must be carefully collected and assessed. Keener tools for diagnosis and treatment have increased the interest of the private physician in tuberculosis. Our educational program may need to be recast for this receptive audience. The general practitioner and the specialist in internal medicine can today, more than ever, participate in the antituberculosis campaign. We, in turn, must cope with the problems of case reporting and case and contact supervision which arise when patients are under the general management of individual physicians.

Many other changes have taken place during the past decade. Most significant—in terms of our future in public health—is the apparent trend of tuberculous disease itself. For many years we have, unquestionably, been able to demonstrate the effectiveness of our case-finding and treatment efforts. In every recent year a dramatic decline in tuberculosis mortality has been apparent. With each year's report of mortality we have, at one and the same time, presented a magnificent record of achievement and forecast growing difficulties for ourselves in maintaining the offensive against tuberculosis.

When the tuberculosis death rate moved from 50 per 100,000 population to 45, the report bespoke a thrilling achievement. Yet, at the same time, the lower level of mortality was still sufficiently high to serve as eloquent evidence—and self-sufficient evidence—that the disease remained a serious public health problem.

Today, with the latest available report of tuberculosis mortality at an estimated 12.6 per 100,000, direct and compelling evidence of the true extent of the problem is not so readily available. This current estimated death rate and a mortality decline of 70 per cent in the last ten years have made justifying control budgets more and more difficult.

[1] From the Division of Special Health Services, Public Health Service, Department of Health, Education, and Welfare, Washington 25, D. C.

[2] This address was delivered at the Fiftieth Anniversary Meeting of the National Tuberculosis Association, Atlantic City, New Jersey, on May 18, 1954.

These two facts are so dramatic that—unfortunately—they overshadow such things as: the 1,200,000 people who have some form of tuberculosis; the 250,000 persons who now are on our case loads as active cases of tuberculosis; the 86,000 persons who fell ill and were added last year to our case load of active tuberculosis; and an estimated 150,000 as yet undetected active cases. Indeed, these morbidity figures raise the question of whether, in fact, we are (as suggested by recent mortality figures) reaching the irreducible, hard-core, minimum level.

While we are confronted, then, with questions regarding the job we have done in the past and the methods we have used to achieve the particular goals of each of our specialized fields of tuberculosis control, we face pressing questions regarding future activities, relative emphases, and operating methods. In this light, let us examine our case-finding efforts of the past, and deal with them in terms of basic public health philosophy and specific objectives. Let us examine them, too, in terms of what implications they may have for the future.

Underlying every case-finding effort, particularly in a communicable disease, is the question of its yield of unknown cases and the question of its value as a preventive measure. A case-finding procedure for tuberculosis must reveal those persons who unwittingly and without suspicion are broadcasting the disease; it must discover the disease process at a point where medical service can result in arrest and cure.

What, then, in general terms, is the essence of our experience? Case finding for tuberculosis is directed toward three distinct groups of people: those who are sick or who have medical complaints—such as we find in clinics, hospitals, and doctors' offices; those who have had tuberculosis or who have had contact with the disease—quite a separate group from those with complaints; those who are apparently healthy—found either in selected groups in a community or in the total community itself.

Our approach to tuberculosis case finding for the first group is conducted through roentgenographic examination of clinic patients, patients admitted to hospitals, and patients of private physicians; in general, the sick and the infirm. They have symptoms and they visit their physician or clinic to find out "what ails them." This kind of case finding has been carried out by physicians for years, and more recently has been extended to include hospital admission roentgenographic programs. The method will be used for years to come. It can be broadened and its performance improved. Even today, a large proportion of patients with active tuberculosis are discovered because of symptoms. Two recent studies illustrate this point: In Iowa, almost three-fourths of the patients admitted to two hospitals sought medical care because of symptoms (1). In Hawaii, one-third of the active cases of tuberculosis reported were discovered because the patient had symptoms (2). In another instance, a general practitioner, in a six-month period in his office practice, discoverd, by careful attention to the complete examination of certain of his patients, 4 cases of active advanced tuberculosis. This was more than 4 per cent of the persons whom he examined roentgenographically (3).

Logically, the yield must be high among the sick and the infirm. Data avail-

able from routine hospital admission programs give substance to this generalization. In such programs, as many as 4 active cases of tuberculosis are reported among every 1,000 persons examined (4).

The second group for case finding—the supervision of both inactive cases of tuberculosis and the contacts of all cases—is traditional to official health agencies.

It is a truism that more active tuberculosis is found among the families and associates of known cases of tuberculosis than among general population groups. A considerable amount of active tuberculosis can be found among family contacts by realistic and conscientious follow-up: from 44 to 53 per 1,000 contacts followed (5, 6). I expect that the rate at which cases of tuberculosis develop among contacts will remain relatively constant in the future. And as the prevalence of tuberculosis in the general population goes down, the relative yield of this procedure will increase. Thus, contact examination will continue as a basic feature of our program.

As for the follow-up of questionably active and inactive cases, we have found this to be one of the most effective ways of detecting active tuberculosis. In recent studies in Iowa, up to 7 per cent of active cases discovered resulted from the follow-up of cases previously classified as inactive (7).

While we have debated how much of our resources for control should go into follow-up of known cases, we must be impressed with the fact that in Hawaii almost one-fourth of the active cases which were known to the health department between 1947 and 1953 resulted from the follow-up of cases initially reported as inactive or questionably active (2). How, then, can we do other than predict and urge the continuance in the future of the supervision of inactive cases and the contacts of all cases?

Now let us turn to our last category—the survey of apparently healthy population groups. This category consists of two parts:

One part—the examination of segments of the population—is dictated by the following general considerations: (*a*) a limitation of case-finding resources and (*b*) the practical matter of organizing a group of people to be examined. It is these two considerations which may result in "pin-pointing" case finding so that the yield of cases may be high per unit of cost.

Thus, we work in those groups among whom the risk of tuberculosis is great, or in those areas in which high prevalence is concentrated by reason of general social and economic conditions. Many students of the subject have characterized as high prevalence groups the population of institutions, the aged, nonwhites, migrant workers, and residents of certain census tracts or other identifiable subdivisions of larger geographic areas. Nonwhites, for example, have roughly twice as much active tuberculosis as white persons.

Inmates of institutions are often more likely to have active tuberculosis because of their previous social status and living habits. They are also likely to acquire tuberculosis from close contact in the institution. Among inmates of mental hospitals, the prevalence of undiscovered active tuberculosis is particularly high, being in the neighborhood of 30 per 1,000 inmates on first examination (8).

We also work with easily organized groups—such as industrial workers, school children, and business or government organizations. Because these populations are concentrated, fairly large numbers can be reached quickly and at relatively low cost. And, in many areas, local ordinances relating to the use of health cards make such employee groups as foodhandlers and domestics quite easy to reach for examination purposes. On the whole, these groups yield about as much tuberculosis as general population surveys yield.

Another one of the productive sources of active cases of tuberculosis is the aged. As I will illustrate later, the prevalence of tuberculosis increases with age, so that case-finding opportunities among the elderly should never be overlooked.

In general, we have found that the prevalence of tuberculosis varies with residence. You are all familiar with the map that every health department has, showing the areas where cases of tuberculosis are concentrated. In these areas, we can generally find most encouraging yields of new hidden cases—in sufficient number to perpetuate indefinitely infection within themselves and among neighboring areas—unless they are detected.

The second part of our case-finding efforts among the apparently healthy is the survey of the total community—or as much of it as is possible in this imperfect tuberculosis worker's world.

Because the prevalence of tuberculosis is higher in metropolitan areas and because local case-finding resources were inadequate to deal with such population masses, the Public Health Service assisted in taking eight million roentgenograms in twenty-five large city community-wide programs from 1947 to 1953. This total of eight million roentgenographic examinations represented some 70 per cent of the population fifteen years of age and older in the areas involved. In these programs, we labeled approximately one person per 1,000 persons examined as an active tuberculosis case, and 6 persons per 1,000 examined were classified as having inactive disease. From 85 to 90 per cent of these cases were unknown to health authorities.

By way of further illustration of the potential yield of the mass survey, I should like to cite the experience of upper New York State in using this approach to case finding. During the five years from 1947 to 1952, chest roentgenograms were obtained on almost one million persons. Approximately 1.3 new active cases of tuberculosis were discovered for each 1,000 persons examined (9).

However, in other areas roentgenographic examination of whole population groups has been less productive. In Iowa, for instance, mass roentgenographic surveys in nonurban counties produced only about one case of new active tuberculosis for every 8,000 persons examined in 1952.

Thus we see that, although the yield from mass roentgenographic programs will average about one active case per 1,000, the yield will vary from area to area.

Let us turn now to the use of the tuberculin test as a mass case-finding device among apparently healthy population groups. (I am not referring now to the use of the tuberculin test as an index of the infection level in a community.) Actually, tuberculin testing of whole communities or selected populations does not differ in principle from mass roentgenographic examinations with the use of the 70 mm. film. Only the mechanics differ. The decisions important to the

treatment and control of tuberculosis depend upon the roentgenographic and bacteriologic findings. In those groups among whom the percentage of tuberculin reactors is, say, 5 per cent—as among school children, for example—it may be advisable to choose the tuberculin test as the primary case-finding mechanism in preference to the roentgenogram. However, in groups among whom tuberculin reactions will total a larger percentage (and this might be encountered where older persons comprise an appreciable proportion of the population to be examined), it is probably preferable to use the 70 mm. film as the detecting device.

One of the advantages of the tuberculin test is the simplicity of the equipment. The test is easy to apply, especially in rural areas or among groups of people difficult to reach for a roentgenogram. The tuberculin method is used among pre-school and school children. An elaboration of this method is the examination of the contacts of positive reactors. That the yield reported from tuberculin testing varies, I can illustrate by several examples. In the District of Columbia, a sample of 3,000 children were tuberculin tested this past year to establish the tuberculin sensitivity levels in the District. Three of the children who reacted were found to have healing primary tuberculosis. A follow-up of 380 contacts of the positive reactors produced 4 persons in whom tuberculosis was classified as active or activity undetermined. In Wisconsin, tuberculin testing of 36,000 children produced 18 active cases of tuberculosis, or 0.5 cases per 1,000 examined (10). In general, few data are available regarding the specific contribution of follow-up of contacts of positive reactors.

Recently much thought has been given to tuberculin testing of whole communities, especially in low prevalence areas. Eighty per cent of the population in two counties in Minnesota were tested. In all, approximately 20 per cent reacted positively. All positive tuberculin reactors were examined roentgenographically. No active cases of tuberculosis were identified (11).

So much for the relative productivity of the various general approaches to tuberculosis case finding. Yield alone, however, is by no means the sole criterion of the efficacy of a case-finding technique. The quantitative standard is important, to be sure, especially in these days of straitened tuberculosis control budgets.

As practitioners of public health and preventive medicine, however, we must, at the same time, evaluate the various case-finding techniques on a qualitative basis. In specific terms what kinds of cases do each of our case-finding programs find—and of what relative significance is their discovery from the standpoint of public health and preventive medicine?

Logically, the greatest benefit derives from early discovery. This is especially true today, considering the availability of modern drug and surgical therapy.

With early discovery as our touchstone, let us look now at the qualitative results of case finding in the three groups.

Among the first two groups—those with medical complaints, and those who represent cases and contacts—we find that yield, in terms of numbers of cases found and in terms of case-finding rates, will perhaps be better than for the mass approach. However, the kinds of cases found will by and large be at a later stage of disease when health benefits are not so great.

As I have indicated previously, chest roentgenograms among hospital admissions are profitable and practicable, discovering from 2 to 4 active cases for every 1,000 persons examined. In two fairly recent studies, one in New York State and one in New Orleans, the proportion of active cases of tuberculosis found in the minimal stage was smaller than the proportion found in community-wide chest roentgenographic surveys (12). This might well be expected in view of the fact that the people admitted to the hospital are sick people. It is quite probable that in some instances their illnesses were related to tuberculosis. It seems quite likely then that patients with tuberculosis found in hospital admission programs have already broadcast their disease to a somewhat greater extent than patients found when more aggressive measures are applied. The same can certainly be said for clinic patients and patients in private physicians' offices.

Of the first two groups, I believe the supervision of contacts and inactive cases offers the most promise of early detection. If we examine these groups frequently, every new case of tuberculosis which might arise among them will be discovered at the point of conversion to activity. This, therefore, is a case-finding procedure which no official health agency should overlook.

We must acknowledge the fact that case finding among contacts and inactive cases is of practical necessity relatively limited in terms of numbers of people served. And, although the case-finding rate itself can be high, the total number of cases found will be relatively few. Moreover, since inactive cases are neither reported nor supervised in many parts of the country, early case finding among this group will not be accomplished without changes in administrative procedure.

As to surveys among special segments of the apparently healthy population or specific geographic areas, we have also found that the kinds of cases discovered in populations with high yields frequently tend to be at a more advanced stage than those found in groups with a lower prevalence. As I mentioned earlier, the prevalence of tuberculosis is greatest in the older age groups. However, cases discovered in this group also tend to be more advanced.

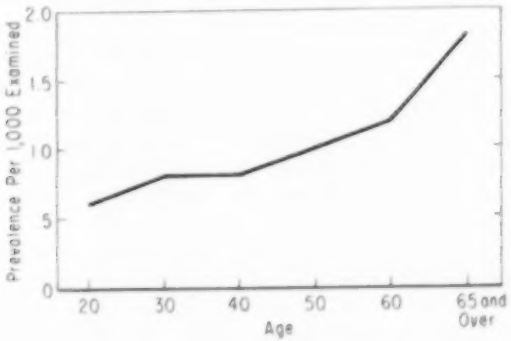


FIG. 1. Prevalence of active tuberculosis by age; three community-wide roentgenographic surveys, 1948-1949.

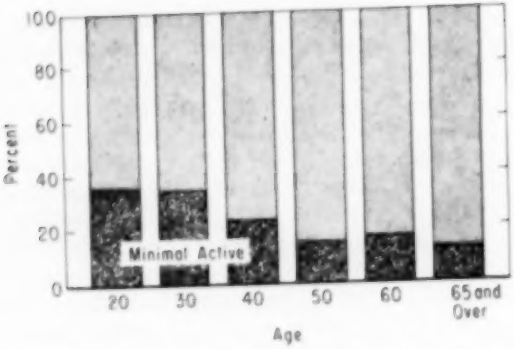


FIG. 2. Minimal active tuberculosis as percentage of total active tuberculosis by age; three community-wide roentgenographic surveys, 1948–1949.

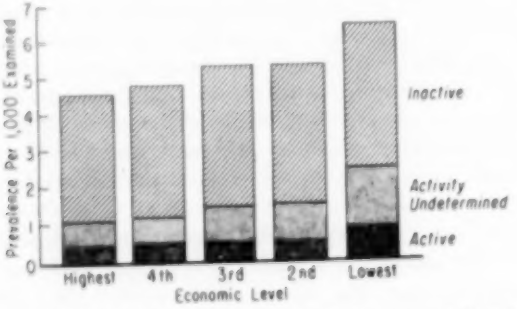


FIG. 3. Prevalence of newly discovered tuberculosis by economic level; Cleveland, Ohio roentgenographic survey, 1949.

In three community-wide surveys, the percentage of active tuberculosis found in the minimal stage varied from 35 per cent in the age group fifteen to twenty-four to only 13 per cent in the age group sixty-five and older.

We have recently been studying the yield of tuberculosis found in relation to the economic status of the participants in one of the community-wide programs in which we worked (13). We arranged the census tracts of the city into five economic groups according to family income. The census tracts in the lowest income group were those we traditionally think of as high prevalence areas on the basis of mortality and on the basis of routine case reporting. In fact, 60 per cent of the tuberculosis deaths come from the 20 per cent of the population in this lowest income area.

In the low economic group, 0.9 persons with active tuberculosis were found for every 1,000 examinations as compared with slightly more than 0.4 per 1,000 in the highest. From this it would definitely appear that the most productive place to do case finding for active tuberculosis is in the low economic group and the low economic area. By stage of disease, however, the percentage of cases

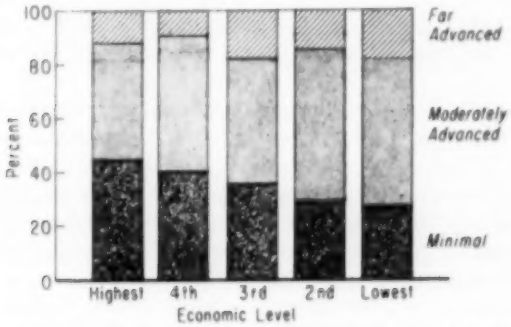


FIG. 4. Stage of disease of newly discovered active tuberculosis by economic level; Cleveland, Ohio, roentgenographic survey, 1949.

that were minimal varied systematically, with the economic level ranging from only 27 per cent in the lowest economic level to 45 per cent in the highest. Conversely, the percentage of the far advanced stage ranged from a high of 18 per cent in the lowest economic group to only 11 per cent in the highest. This strongly suggests that the disease was intercepted at an earlier point in the higher economic group, and it seems to me that we would be remiss to compare the absolute yield of these various economic groups without considering this important factor.

Moreover, I should like to point out that, if the chest roentgenographic survey in this city had been concentrated only in the lowest economic group and had succeeded in completely examining that group, it is probable that two-thirds of the unknown active tuberculosis in the city would have been missed. Of all the undetected tuberculosis in the area—that is, both active and inactive disease—the lowest economic group would have produced only one-quarter of the cases.

Since our major concern, then, is with early discovery, it will be well for us to look for a moment at the experience of Minneapolis, Minnesota, following its 1947 community-wide chest roentgenographic survey.

A four-year follow-up of active cases discovered before and during the survey (14) showed that 37 per cent of the survey cases were discovered in the minimal stage, as compared with only 22 per cent of nonsurvey cases. Sixteen per cent of the survey cases were in a far advanced stage, as compared with 33 per cent of nonsurvey cases. A life-table method was used to study the survival of these two groups of cases during a four-year period. It was estimated that the chance that a survey case would prove fatal within four years was one of 10, while the chance of a nonsurvey case being fatal was one of 3. The two groups compared were quite similar in terms of age, sex, race, and bacteriologic status. I can only conclude in the light of these data that the differences are a reflection of the value of early case discovery through intensive case-finding efforts.

For the examination of apparently healthy populations, then, we find that, although the yield may be relatively low in comparison with case finding among

the other groups, the cases discovered are more likely to be at an early stage of disease—when the potential benefit to the patient and to the public health is greatest.

On closer examination of the mass roentgenographic technique, one further significant epidemiologic fact comes to light. We have found that a great many of the patients with active disease present no symptoms at all and that, among those who do present symptoms, these are very often not severe enough to bring them to medical attention without special case-finding efforts.

From the foregoing, I think you will agree that the examination of apparently healthy people is an important part of the control of tuberculosis, and that comparison of the productivity of the various types of case-finding efforts requires more than the examination of the yield in terms of numbers or rates alone.

I believe that examinations of apparently healthy populations must be conducted on a periodic basis. If these examinations are confined to single efforts, as was the tendency in the recent community-wide chest roentgenographic programs, their impact on the tuberculosis problem is only slight. Let me give you some actual data to demonstrate this point. In Seattle, Washington, we have studied the reported incidence of active tuberculosis prior to, during, and after a community-wide chest roentgenographic survey (15). In doing so, we found that active tuberculosis discovered as the result of the roentgenographic survey was only a small proportion of all active tuberculosis reported during a three and one-half-year period, despite the fact that excellent participation was achieved. The impact of the survey on reported incidence for a period of years was probably not great. The only noticeable decline occurred immediately following the survey. In other words, the probable net effect of a single mass roentgenographic survey program is to modify only slightly the trend of tuberculosis incidence in the area covered.

In studies we have been conducting in Columbus, Georgia, on the occurrence of tuberculosis, it has been our conclusion that roentgenograms should be taken at frequent intervals if any appreciable portion of all cases is to be detected early and if the normal trend of tuberculosis incidence is to be affected permanently.

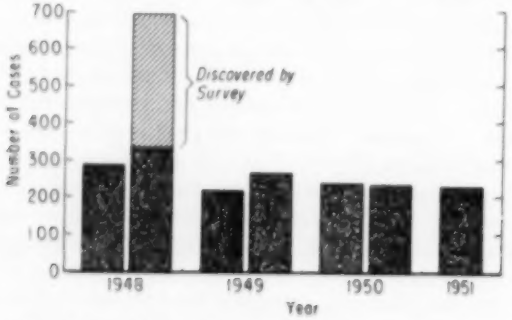


FIG. 8. New active tuberculosis; Seattle-King County Health Department, semi-annually, 1948–1951.

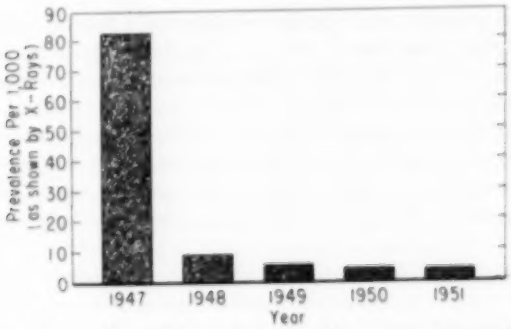


FIG. 6. Decline in new tuberculosis suspects found in repeated surveys (9 mental hospitals in California).

Unfortunately, or fortunately, the type of program I am proposing quickly results in an extremely low yield per roentgenogram taken. In a roentgenographic program in nine mental hospitals, reported by the state of California, in which institutional populations were resurveyed for a number of successive years, the yield of undetected tuberculosis dropped very rapidly with each case-finding effort, gradually leveling off (16). Mental hospitals, of course, represent a partly "closed" population, and one might expect the rate to fall here more quickly than in a general community because of the problem of migration and of incomplete participation in community roentgenographic survey programs. We are convinced, however, that the greatest good can be obtained only through repeated case-finding programs on a mass basis.

In the light of present conditions, this conviction leaves us on the proverbial horns of the dilemma. Carrying out such a recommendation, we will find ourselves faced with progressively decreasing yield and concurrently increasing cost per case discovered—a most serious consideration. In short, we should soon find ourselves in the position of being forced to abandon case finding among apparently healthy people completely because of its continued great cost and low yield. Thus, we would allow prevalence of undiscovered disease slowly and surely to return to a higher level. This, I think we can all agree, would be disastrous at this time of near realization of our ultimate objective. It would be even more disastrous in view of the fact that we now, for the first time in the history of tuberculosis control, have effective means of treating the discovered cases of tuberculosis. It would indeed be a tragedy—for both the individual patient and for society—if we were unable to make full capital of this new factor in tuberculosis control.

To sum up, we see that case finding in the future requires:

1. The examination of the sick and the infirm in clinics, hospitals, and the doctor's office
2. The follow-up of inactive cases and contacts of cases
3. The periodic examination of apparently healthy people

This last, the periodic examination of apparently healthy people—the community-wide case-finding technique—is, in my opinion, a necessary method of case finding. However, it has been demonstrated that such periodic examinations result in an increasingly low rate of yield or, in other terms, a high cost per case found. This is so, no matter whether the roentgenogram or the tuberculin test is the method of examination. Ultimately, this cost will prohibit mass case finding. It is necessary, therefore, that we seek ways in which tuberculosis case finding in apparently well groups can be accomplished without unreasonably high cost.

It seems to me that the future of tuberculosis case finding in apparently well people does not lie in the use of the roentgenogram or tuberculin, nor will it be found in any other test which will detect only tuberculosis. Ultimately, high cost and low yield will relegate such a single-purpose test to medical history. We in tuberculosis work have played an important role in the evolution of modern techniques for the detection of tuberculosis. It would be most appropriate, therefore, for us to encourage a conscious and vigorous search for new techniques which can be applied easily and economically in the detection of more than just the one disease of tuberculosis. In doing so, we should be sensitive to the growing health needs and interests of our people.

While support for mass surveys for tuberculosis will wax and wane with the prevalence of the disease, interest is increasing in mass surveys for heart disease, cancer, kidney disease, neurologic disorders, disturbances in metabolism, and other diseases which are afflicting our people. For these people, like the tuberculous patient, early diagnosis holds the same promise of early treatment and early arrest or cure.

In exploring this area of future activity for tuberculosis case finding, I am not contemplating multiple diagnostic testing as we know it today. For this procedure relies on a battery of tests, most of them specific for single diseases only. Since each test adds its individual cost, the process already is unavoidably costly.

In most past efforts at developing diagnostic tests, we have strained at eliminating false positive reactions and decreasing the false negative yield for a specific disease. In many instances these so-called false positives are found to be due to other diseases. Now I would propose that our effort go toward developing either a single test or a few tests that would identify all of the people who might have any of a whole group of diseases, including tuberculosis. For those reacting positively, the determination as to which specific disease and body system is involved would require secondary tests to confirm the finding and narrow the reasons for referral to physicians for clinical study.

If we can, for example, find a primary test which will be sensitive to physiological disturbances in general, we will have taken a giant stride forward—not only for future progress in the control of tuberculosis. We will have taken a giant stride forward in our efforts to control the major diseases of man.

While I am obviously and wishfully speculating on, and hoping for such a test, I can quickly give you familiar examples of what I mean. A questionnaire has been developed, known as the Cornell Medical Index. On the basis of the replies

which a person gives about his past and present health history and symptoms, a crude separation of the healthy can be made. Of course, we say, this has the disadvantage of being subjective and dependent upon symptoms which we think of as occurring slightly late in a disease process. Then there is the erythrocyte sedimentation test. This test is positive in a great many conditions, both normal and abnormal. It is positive in both active tuberculosis and the common cold. Its present defect is that it is too sensitive. But these tests have a spectrum much broader than the chest roentgenogram. Actually, we have thought of the chest roentgenogram as a broad test in the examination of apparently healthy people, compared with, for example, the tuberculin test. There are almost one hundred conditions which can be seen on a chest roentgenogram—the most common and important of which are tuberculosis, heart disease, and lung cancer. Some of our roentgenographic programs have pointed out the application of the roentgenogram to the latter two problems. But controversy surrounds the question of its value in searching for these diseases and I think we must recognize for the present that the roentgenogram as a mass detecting device must of necessity relate primarily to the search for tuberculosis. I do not think the chest roentgenogram as we know it will be used extensively in mass surveys when the yield of tuberculosis becomes low.

I am thinking of and (as I have said earlier) hoping for a single yet-to-be-developed test which will be used periodically for a broad spectrum of diseases, including tuberculosis, at low cost and productive yield.

In practice, such an omnibus test would be applied as a primary detecting device to be followed by secondary tests (such as roentgenograms and tuberculin testing) to inquire into the tuberculous nature of the disturbance. The most practical implications of such a test would be that it would provide a means for assured continued efforts until the last case of tuberculosis can be found.

At the present time, a test of this particular kind is not wholly visionary or entirely in the realm of science fiction. Dr. Andrew H. Dowdy's test for cancer, a relatively simple and inexpensive blood test, shows promise of being sensitive not only to cancer but possibly to other diseases as well.

Another test is the lipase index of tuberculosis which has been developed by Dr. Paulo Seabra of Brazil. This test is now under study and, should final evaluation substantiate its present promise, we will at the very least have a blood test for tuberculosis at a stage prior to the existence of clinical manifestations. This test, too, gives indication of applicability to more than the one disease.

* * *

The form of tuberculosis control of tomorrow is being carefully weighed in the balance today. The search for the tuberculous sick and infected must continue unabated until tuberculosis itself has been finally relegated to medical history. For the time being, we must continue to use the tools at hand as vigorously and as aggressively as practical considerations will allow.

The mass survey techniques and programs of today and tomorrow are totally dependent on how well we follow through in getting the people whom we find to

doctors, where complete and thoroughly serious probing of the findings of the survey will be done. This can be our effort today in our daily work. At the same time, we must encourage and actively join in the search for the omnibus type of case-finding device through which we can assure continued effort and sustained progress against tuberculosis.

Such a device, coupled with the newer and infinitely more effective methods of treatment which tomorrow will surely bring, will give to us that ultimate victory over tuberculosis which we have sought for fifty years today.

REFERENCES

(1) Unpublished data from Iowa; from table, "Reasons for Coming to Diagnosis," dated March 25, 1954.

(2) Unpublished data from study of Hawaii tuberculosis case register.

(3) Frohman, I. P.: Tuberculosis survey and the general practitioner, M. Ann. District of Columbia, 1953, *22*, 133.

(4) Routine chest X-rays of all patients admitted to general hospitals, Pub. Health Rep., 1954, *69*, 569.

(5) Puffer, R. R., Zeidberg, L. D., Dillon, A., Gass, R. S., and Hutcheson, R. H.: Tuberculosis attack and death rates of household associates, Am. Rev. Tuberc., 1952, *65*, 111.

(6) Telford, P. K., and Garten-White, R.: Spread of tuberculosis in families of tuberculous patients, Am. Rev. Tuberc., 1946, *53*, 215.

(7) Anderson, R. J., Galinsky, L. J., Gelperin, A., and Iskrant, A. P.: Trends in discharge and length of stay of patients in a tuberculosis hospital, Pub. Health Rep., 1954, *69*, 781.

(8) Anderson, R. J.: Tuberculosis problems in mental hospitals; reprints of papers of psychiatrists, public health physicians, and internists published in the March, April, and May, 1952, issues of *Mental Hospitals*.

(9) Siegal, W.: The first million chest X-rays, *Health News*, New York State Department of Health, 1953, *30*, 9.

(10) Pleyte, A. A., Kerwin, D., and Sternitzky, D.: The value of tuberculin skin testing as a case-finding procedure, Journal Lancet, 1953, *73*, 130.

(11) Annual report of the Minnesota Tuberculosis and Health Association for the year April 1, 1951, to March 31, 1952; and personal communication dated April 28, 1954, from S. A. Slater, M. D., Superintendent and Medical Director, Southwestern Minnesota Sanatorium, Worthington, Minnesota, to Paul Pamplona, M. D., Division of Special Health Services, U. S. Public Health Service.

(12) *a.* Siegal, W.: Chest X-rays of general hospital admissions, *Health News*, New York State Department of Health, 1950, *27*, 3.

b. Jacobs, S.: Routine photoradiography in a general hospital, Journal Lancet, 1952, *72*, 189.

(13) Anderson, R. J., Enterline, P. E., and Turner, O. D.: Undetected tuberculosis in various economic groups, Am. Rev. Tuberc., 1954, *70*, 593.

(14) Anderson, R. J., Enterline, P. E., Hill, F. J., and Roberts, J.: An evaluation of tuberculosis detection by chest X-ray surveys, Pub. Health Rep., 1954, *69*, 1053.

(15) Data from the King County, Washington Health Department, tabulated by Miss Sara Pearson of the Division of Special Health Services, U. S. Public Health Service.

(16) Crawfis, E. H., Kupka, E., and Oechsli, W. R.: Tuberculosis control in California mental hospitals, California Med., 1953, 79, 390.

INVESTIGATION OF IRREGULAR DISCHARGE OF TUBERCULOUS[1] PATIENTS

Report of a Preliminary Study

THOMAS H. LORENZ, WILLIAM C. LEWIS, GEORGE CALDEN, AND JOHN R. THURSTON

(Received for publication May 26, 1954)

INTRODUCTION

To professional persons not concerned with the treatment of tuberculous patients, irregular discharge[2] is a rare and curious phenomenon. To the tuberculosis specialist, however, irregular discharge is a very real and chronic problem. It has been reported that the average irregular discharge rate from larger tuberculosis hospitals approaches 50 per cent (1). This observation suggests that the current therapy program for tuberculosis unfortunately is designed for but half of the persons who contract this disease. It also suggests that, no matter how effective chemotherapy and surgery may be theoretically, their ultimate value will be limited until a greater proportion of patients follow treatment to completion.

Although theories and opinions are legion, the precise causes of irregular discharge are not known. In recent years, the role of situational and emotional maladjustment has been stressed (2–5). At the same time the significance of such factors as age, race, marital status, extent or duration of disease, et cetera has been largely discounted. The reports of Tollen (1) and Leibovici (6) provide some basis for this opinion. Nevertheless, in the absence of a controlled study, in which large, unselected series of regular and irregular discharge patients are compared in terms of such variables, the lack of significance of these factors has not been definitely established.

In the present study, 100 consecutive patients who received irregular discharges have been compared with 100 consecutive patients who received regular discharges. In so doing, it was believed that the significance of the aforementioned factors (and other similar ones) could be established with reliability.

This report covers one portion of the preliminary phase of an investigation of irregular discharge which is being conducted at the Veterans Administration Hospital, Madison, Wisconsin. Other aspects of this investigation, particularly those having to do with the factors of situational and emotional maladjustment in irregular discharge, are being published separately (7).

[1] From the Psychomatic Section of the Medical and Tuberculosis Service, Veterans Administration Hospital, Madison, Wisconsin.

[2] Irregular discharge is defined as "any termination of hospitalization of a living patient which is not medically sanctioned by professional authority" (1). There are three categories of irregular discharge: leaving against medical advice; absent without official leave; and disciplinary discharge.

TABLE 1

HOSPITAL CENSUS AND DISCHARGES BY MONTHS

Month	Average Daily Patient Load	Irregular Discharges	Regular Discharges
September, 1951	4	0	0
October, 1951	15	0	0
November, 1951	48	1	0
December, 1951	77	1	0
January, 1952	90	3	2
February, 1952	127	6	0
March, 1952	141	5	2
April, 1952	172	12	3
May, 1952	178	6	4
June, 1952	201	3	5
July, 1952	215	7	6
August, 1952	250	8	8
September, 1952	267	11	8
October, 1952	266	7	17
November, 1952	268	5	3
December, 1952	282	11	15
January, 1953	305	5	10
February, 1953	308	12	12
March, 1953	305	14	14

MATERIALS AND METHOD

General

The Veterans Administration Hospital, Madison, Wisconsin, was opened on September 15, 1951. This institution, which is restricted to the treatment of tuberculous patients, is operated as a Deans Committee Hospital in collaboration with the University of Wisconsin Medical School.

During the first eighteen months of operation, the hospital population rose steadily (table 1) until January, 1953, when the daily average census was just over 300 patients. In this period, 109 patients were discharged as having obtained maximal hospital benefit and 117 received irregular discharges.

Subjects

The first 100 subjects in both discharge groups were included in this study. The patients were selected solely on the basis of two criteria: (*1*) the type of discharge given them, and (*2*) their proved diagnosis of tuberculosis. Since each of the first 100 patients in the irregular discharge group had an established diagnosis of tuberculosis, none was excluded from the series. On the other hand, 9 patients who received regular discharges were not included in the study.[1] These persons were admitted for observation and then found to have no tuberculosis.

The first 100 irregular discharges were of the following variety: 81 against medical advice, 10 absent without official leave, and 9 disciplinary discharges.

Collection and Analysis of Data

Data pertaining to age, race, marital status, occupation, patient distance from home, military service connection (tuberculosis), extent of tuberculosis, activity of tuberculosis,

[1] Patients who receive "regular" discharges via transfer, for administrative reasons or because of death, are *not* included in the category of regular discharges.

pulmonary surgery, length of hospitalization, and history of previous irregular discharge were collected from each patient's hospital record.[4]

The significance of differences between the two groups of patients in regard to each of these factors was determined statistically by using the chi-square test.

RESULTS

Nonsignificant Factors

Statistical comparisons between the two groups on the basis of age, race, marital status, patient distance from home, military service connection for tuberculosis, and activity of tuberculosis revealed no significant differences (table 2).

Significant Factors

Occupation: Statistical analysis of the data on occupation demonstrated that the two groups differed significantly at the 0.05 level of confidence. Inspection of the data (table 3) shows that this difference is largely attributable to the 12 patients who had no known occupation and to the preponderance of semi-skilled laborers among patients who received irregular discharges in comparison with those who received regular discharges. In the other classifications there is marked similarity between the two discharge groups.

Pulmonary surgery: As shown in table 3, fewer patients in the irregular discharge group had surgical treatment during their hospitalization than did the patients in the regular discharge group. Statistically, this difference is significant at the 0.06 level.

Length of hospitalization: The mean length of stay for the patients who received irregular discharges was 148 days; whereas, for those who received regular discharges, it was 260 days. The difference between means of 112 days proved to be statistically significant at the 0.01 level of confidence. The period of hospital stay prior to irregular discharge ranged from less than one day to nearly twelve months, while the range before regular discharge was from twelve days to almost sixteen months.

Although irregular discharges occurred at any time in the course of hospitalization, 40 per cent were within the *first three months* after admission and only 8 per cent took place after 300 days. In contrast to this, only 15 per cent of the patients in the regular discharge group left within three months, and more than 40 per cent of them were hospitalized for 300 days or more before discharge (figure 1). The data in table 4 demonstrate the wide range in hospital stay prior to discharge and also show the notable difference in length of hospitalization between the two groups of patients in the series.

Extent of tuberculosis: Figure 2 makes apparent the striking similarities and differences in terms of the extent of their tuberculosis between the series of patients who received irregular discharges and those who received regular

[4] The data relating to extent and activity of tuberculosis were obtained from the records of the *first* (admission) medical evaluations of the patients by the hospital staff. The data on pulmonary surgery applied only to thoracic surgery performed during the patients' *current* hospitalization.

TABLE 2

COMPARISON OF 100 IRREGULAR DISCHARGE PATIENTS WITH 100 REGULAR DISCHARGE PATIENTS

Age (Years)	Irregular Discharge Patients	Regular Discharge Patients
Less than 20	0	0
20–29	32	31
30–39	23	25
40–49	12	7
50–59	22	29
60–69	10	8
70–79	1	0
Race		
White	79	84
Negro	17	13
Other	4	3
Marital Status		
Married	56	56
Single	29	31
Divorced	12	8
Widowed	3	5
Military Service Connection (Tuberculosis)		
None	64	66
50–100 per cent	32	34
Unjudicated	4	0
Activity of Tuberculosis		
Active	76	69
Inactive	19	28
Undetermined	3	0
Nonpulmonary tuberculosis	2	3
Patient Distance from Home (Miles)		
0	7	7
25	1	2
50	4	3
100	16	17
200	49	46
300	11	14
400	6	4
500	5	4
1,000	1	3

TABLE 3

COMPARISON OF 100 IRREGULAR DISCHARGE PATIENTS WITH 100 REGULAR DISCHARGE PATIENTS

Occupation	Irregular Discharge Patients	Regular Discharge Patients
Professional	2	2
Semi-professional	6	7
Managerial	2	3
Student	4	7
Sales-clerical	10	13
Skilled labor	14	21
Semi-skilled labor	38	28
Personal service	10	15
Farmer	2	4
Unknown	12	0
Pulmonary Surgery		
Nonsurgical	64	51
Postoperative	36	49

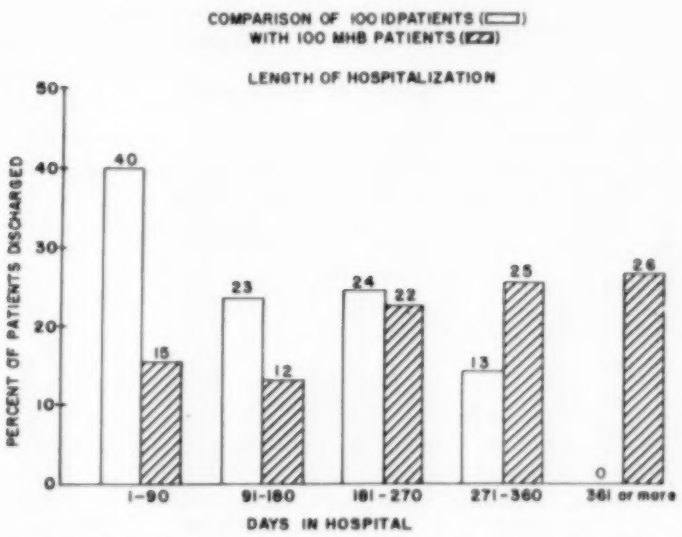


FIG. 1. Comparison of 100 irregular discharge patients with 100 regular discharge patients. Note that with increasing length of hospital stay the number of patients in the irregular discharge group decreases while the number of patients in the regular discharge group increases. Forty per cent of the patients who left the hospital irregularly did so within the first three months, whereas more than 40 per cent of the patients who received regular discharges were hospitalized for 300 days or more.

TABLE 4

COMPARISON OF 100 IRREGULAR DISCHARGE PATIENTS WITH 100 REGULAR DISCHARGE PATIENTS

Length of Hospitalization (Days)	Irregular Discharge Patients	Regular Discharge Patients
1-30	9	6
31-60	15	1
61-90	16	8
91-120	4	6
121-150	6	2
151-180	13	4
181-210	7	6
211-240	11	8
241-300	11	15
301-360	8	18
361 or more	0	26

discharges. The incidence of nonpulmonary tuberculosis and the number of moderately advanced cases were found to be essentially the same in the two groups. On the other hand, in the irregular discharge series, half as many patients had minimal disease and twice as many had far advanced tuberculosis

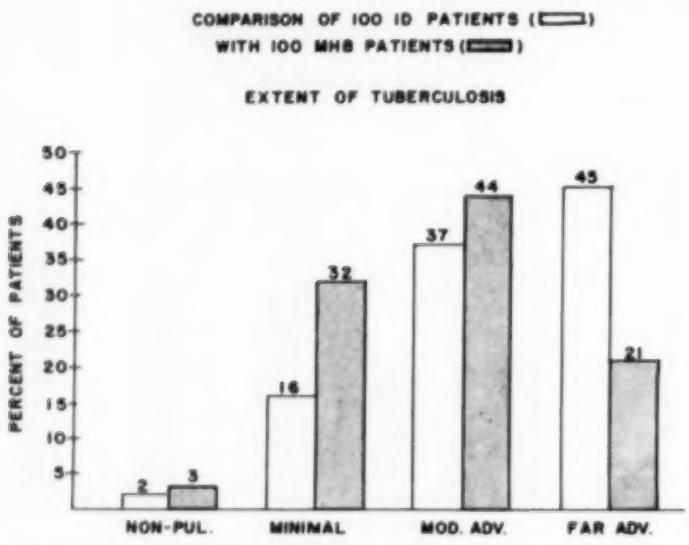


FIG. 2. Comparison of 100 irregular discharge patients with 100 regular discharge patients. Note that, in the irregular discharge group, twice as many patients had far advanced tuberculosis and half as many had minimal disease as the patients in the regular discharge group.

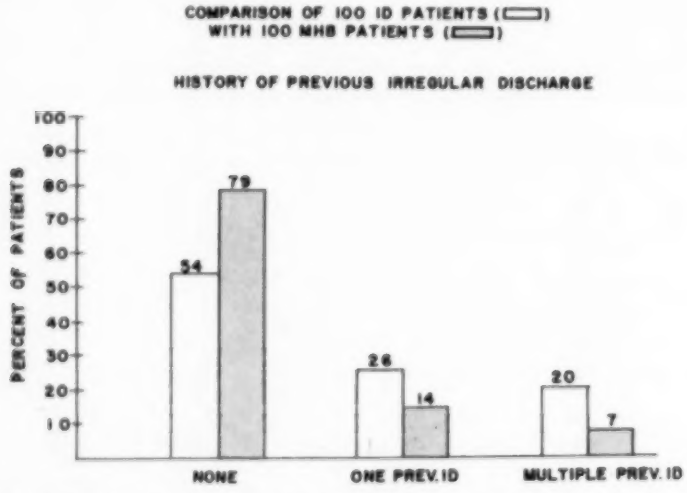


FIG. 3. Comparison of 100 irregular discharge patients with 100 regular discharge patients. Forty-six per cent of the patients in the irregular discharge group had a history of *previous* irregular discharge in comparison with only 21 per cent of the patients in the regular discharge group.

as those in the regular discharge group. On statistical analysis, these differences were demonstrated to be significant ($P < 0.01$).

History of previous irregular discharge: Among the patients who received irregular discharge from their present hospitalization, there was a significantly higher incidence ($P < 0.01$) of *previous* irregular discharge than among the patients discharged regularly from this hospitalization. As shown in figure 3, nearly half (46 per cent) of the irregular discharge group had a history of irregular discharge in the past. Of this number, 26 per cent had one prior irregular discharge and 20 per cent had two or more previous irregular discharges. In contrast to this, only about one-fifth (21 per cent) of the patients with regular discharges had a history of previous irregular discharge: 14 per cent having had one and only 7 per cent having had two or more irregular discharges in the past.

DISCUSSION

From the results of this study, it is apparent that tuberculous patients who leave the hospital irregularly cannot be distinguished from those who leave with official sanction on the basis of age, race, marital status, military service connection, distance from home, or the activity of their disease. The problem of irregular discharge, therefore, would not seem to be that of the young or the old, the white or the Negro, the single or the married, the widowed or the divorced. The fact that a patient is far removed from his home, family, and friends or, on the other hand, the fact that he is near them had no differential significance in the present series. Even the serious consideration of money, in the form of compensation for a military service-connected disability, did not serve to distinguish between the two groups. Furthermore, whether the patient had active disease on admission (and was, therefore, a serious threat to society as well as to himself) or whether he had inactive disease was not found to be a distinguishing factor.

The statistical results which were concerned with the factors of occupation and pulmonary surgery were of borderline significance. The larger number of patients in the irregular discharge group who were classified as semi-skilled laborers may reflect the observation that the type of person who has been referred to as a "muscle man" (7) has a particularly hard time adjusting to the prolonged and passive-dependent type of existence that the treatment for tuberculosis requires. Also, the "drifter" is a notoriously uncooperative tuberculous patient. Thus, it might be expected that more patients who left the hospital irregularly would have "no known occupation."

Why did fewer patients have surgery in the irregular discharge group than those in the regular discharge series? It may be simply that most patients leaving irregularly did not remain hospitalized long enough to warrant surgery. Possibly the increased amount of far advanced disease in the group which received irregular discharge weighed against surgical intervention. Another possibility is that the patients who left irregularly were less willing than the patients in the regular discharge group to accept surgery in much the same manner as they were unwilling to accept prolonged hospitalization. A final explanation for this observation awaits further study.

The finding that patients who leave irregularly remain in the hospital for a significantly shorter period of time than patients receiving a regular discharge is not surprising. This result is as might have been expected, since patients discharged irregularly are not discharged for medical reasons. Instead, they leave the hospital prematurely, against medical advice, or for not returning from pass, or because of disciplinary board action.

Although irregular discharges occur at any time during the course of hospitalization, it is noteworthy that 40 per cent of the patients in this series who received irregular discharges left within the first three months of hospitalization. In Tollen's study of more than 400 Veterans Administration patients who left hospitals through irregular discharge, the same trend was found. He noted that irregular discharge increases with length of hospitalization up to three months and then decreases (1).

The basis for this relationship has not yet been defined, but at least one important and practical implication of this observation is apparent. In most instances, *some* time is available for the detection of potential irregular discharge patients and for the institution of preventive measures when these have been developed. Furthermore, the finding that the majority of patients who leave irregularly do so quite early in the course of their hospitalization indicates that these patients do not leave merely because they have been confined to the hospital for a long period of time. Evidently, the experience of prolonged hospitalization is not responsible for most irregular discharges.

The results relating to extent of tuberculosis (on admission) in the two discharge groups might be construed to mean that far advanced disease provokes irregular discharge. Although this interpretation eventually may prove to be correct, at the present time it is not valid. Among the patients with far advanced disease in the irregular discharge group, there was a considerably higher rate of *previous* irregular discharge than there was among the far advanced patients who received regular discharge. Thus, it may be that the greater number of patients with far advanced disease in the irregular discharge series is more related to an established "irregular discharge pattern" of reaction in these patients than it is to the presence of their extensive disease. Indeed, it might even be that their far advanced disease is a consequence of their previous irregular discharges and not that their far advanced disease provokes irregular discharge.

The finding that 46 per cent of the patients who received irregular discharge had a *past* history of irregular discharge as compared with only 21 per cent of the regular discharge group suggests that, once irregular discharge is resorted to, it tends to repeat itself. The results also demonstrate that a history of *multiple* previous irregular discharges carries with it an even greater probability of subsequent irregular discharge. Most patients who leave irregularly are informed of the dangers of progression of their disease, toxic relapse, infection of children, alienation of family and community, which are in the offing. These consequences are not sufficiently effective, however, to act as deterrents to overcome the forces which make for irregular discharge. In spite of them, irregular discharge tends to be repetitious. It is noteworthy, however, that in these results there also is

evidence of a *counter*-tendency. Irregular discharge patients do not sever their ties with hospitalization and reject it permanently but migrate in and out of hospitals. Thus, the relatively frequent occurrence of multiple irregular discharge can be regarded as evidence that such patients tend to *return*, as well as to leave.

It is encouraging that a few patients with a past history of multiple irregular discharge remained in the hospital on the present admission until they received a regular discharge. These were the exceptions, yet even a few examples of this type indicate that, if irregular discharge has occurred before, it is not certain that it will occur again. Even in the presence of a confirmed "irregular discharge pattern," the situation is not an entirely hopeless one.

If irregular discharge is to be attacked successfully, indices must be developed to permit the early detection of patients with marked "irregular discharge proneness." The present data at least suggest that extent of tuberculosis and history of previous irregular discharge may have value as predictors of future irregular discharge. Currently underway at this hospital is an investigation involving serial admissions rather than consecutive discharges. This method of study should provide valid conclusions regarding the merit of these two factors as indicators of a patient's "irregular discharge proneness."

SUMMARY

The present report is devoted to the results of a preliminary phase of an investigation of irregular discharge in which 100 consecutive patients who received irregular discharge and 100 consecutive patients who were regularly discharged have been compared in terms of age, race, marital status, occupation, patient distance from home, military service connection for tuberculosis, extent of tuberculosis, activity of tuberculosis, pulmonary surgery, length of hospitalization, and history of previous irregular discharge.

Analysis of the data failed to disclose differences between the two groups which were significant at the 0.01 level of confidence except in regard to the following: length of hospitalization, history of previous irregular discharge, and extent of tuberculosis. The patients who left irregularly were found to remain in the hospital for a shorter period of time, to have considerably more far advanced disease, and to have a higher incidence of previous irregular discharge than the patients who received regular discharge.

SUMARIO

Investigación del Alta Irregular de los Tuberculosos: Informe sobre un Estudio Preliminar

Está dedicada la comunicación actual a los resultados de una fase preliminar de una investigación del alta irregular en la que 100 enfermos consecutivos que recibieron esa clase de alta y 100 enfermos consecutivos que fueron dados de alta en forma regular fueron comparados en cuanto a edad, raza, estado conyugal, ocupación, distancia del hogar del enfermo, servicios disponibles para tuberculosis, extensión de la tuberculosis, actividad de la tuberculosis, cirugía pulmonar, duración de la hospitalización e historia de alta irregular anterior.

El análisis de los datos no reveló diferencias entre los dos grupos que fueran importantes a un índice de 0.01 de confianza, excepto con respecto a lo siguiente: duración de la hospi-

talización, historia de alta irregular anterior y extensión de la tuberculosis. Según se observó, los enfermos que abandonaron el hospital irregularmente permanecieron allí menos tiempo, tenían una enfermedad mucho más avanzada y mostraban una incidencia mayor de altas irregulares anteriores que los enfermos que recibieron su alta en forma regular.

RESUME

Investigation sur la libération irrégulière de l'hôpital des malades tuberculeux: Compte-rendu d'une étude préliminaire

Le rapport actuel est consacré aux résultats de la phase préliminaire d'une enquête relative à la sortie irrégulière de l'hôpital et concernant d'une série de 100 patients libérés irrégulièrement de l'établissement hospitalier et une série de 100 patients libérés conformément à la règle. Les deux séries sont comparées en termes d'âge, de race, de conditions matrimoniales, d'occupation, d'éloignement du domicile, de tuberculose contractée dans l'armée, d'étendue du processus tuberculeux, de chirurgie pulmonaire, de durée d'hospitalisation et d'antécédents de libération irrégulière.

L'analyse des données n'a permis de déceler aucune différence significative, à un centième près, entre les deux séries, sauf en ce qui concerne les conditions suivantes: durée de l'hospitalisation, antécédents de libération irrégulière et étendue de la tuberculose. On a observé que, chez les malades sortis irrégulièrement, la durée du séjour à l'hôpital était plus courte, l'affection était considérablement plus avancée et que l'incidence des antécédents de libération irrégulière des établissements hospitaliers était plus élevée que chez les malades libérés conformément à la règle.

REFERENCES

(1) Tollen, W. B.: Irregular Discharge: The Problem of Hospitalization of the Tuberculous, Veterans Administration pamphlet, 10-27, 1948.

(2) Frankel, E.: Against doctors' advice, Bull. Nat. Tubere. A., 1942, *28*, 67.

(3) Bobrowitz, I. D.: Why they leave against advice, Bull. Nat. Tubere. A., 1946, *32*, 151.

(4) Beacham, E. C.: Irregular discharge, Am. Rev. Tubere., 1952, *66*, 213.

(5) Johnson, E. K.: Voluntary discharges from a tuberculosis sanatorium, Am. Rev. Tubere., 1941, *44*, 550.

(6) Leibowici, D.: Clinical evaluation of a rehabilitation program for the tuberculous, M. Ann. District of Columbia, 1952, *21*, 239.

(7) Lewis, W. C., Lorenz, T. H., and Calden, G.: Irregular discharge from tuberculosis hospitals: A major unsolved problem, Psychosom. Med. (in press).

Case Reports

PAPILLOMATOSIS OF THE TRACHEA AND BRONCHI[1]

BURNETT SCHAFF AND ROBERT V. THOMSON

(Received for publication July 20, 1954)

INTRODUCTION

Multiple papillary tumors are rarely found in the trachea and bronchi, and few physicians, therefore, have had the opportunity to study the disease. The condition requires serious consideration, as the relentless recurrence of the tumors leads to severe disability and sometimes death. The wartlike lesions occur and recur in the major bronchi; sometimes tracheal lesions are also present. A few of these tumors have been associated with laryngeal papilloma, but there is often no preceding laryngeal disease.

Two cases of patients recently treated at the Coral Gables Veterans Administration Hospital are presented as examples of multiple bronchial papilloma. One patient showed multiple recurrences over a period of seventeen years; the papillomas were confined to the right main bronchus and right upper lobe bronchus. The other patient developed a laryngeal papilloma, followed five years later by similar masses in the trachea, left main bronchus, and left upper lobe and lower lobe bronchi.

Reports of bronchial papillomatosis in the older literature are difficult to evaluate. Without doubt, some lesions so reported were in reality bronchial adenomas, an entity which was defined only as recently as 1931. Many older reports consisted of descriptions of tumors observed through the bronchoscope, with no histologic details or illustrations. Several cases may be cited which are probably acceptable as examples of the disease under discussion.

Syme (1), in 1927, described a 42-year-old female with multiple polypoid laryngeal tumors who subsequently developed similar lesions in the right bronchus. A 24-year-old male was reported by Zamora (2), in 1931, to have developed multiple papillomas of trachea and left bronchus following removal of a benign laryngeal growth. A similar case was reported by Orton (3) in 1932. Jackson and Jackson (4), in 1932, described tracheal and bronchial papillomatosis. In 1938, Pollock (5) reviewed the literature on inflammatory bronchial tumors, including 27 cases diagnosed at autopsy and 77 by bronchoscopy; of these, only two may be considered as examples of true papillomatosis. In 1939 Patterson (6) reported the case of a 7-year-old male with multiple laryngeal papillomas who later developed similar lesions in the trachea and right bronchus. Buffmire, Clagett, and McDonald (7), in 1950, described a 47-year-old male who, after multiple papillomas had been removed from the larynx and bronchi over a period of many years, developed epidermoid carcinomas of larynx and bronchus twenty-three and twenty-five years, respectively, after the original laryngeal papilloma had been removed. Liebow (8) described bronchial papillomatosis in detail in 1952.

CASE REPORTS

Case 1. The patient is a 57-year-old man who was admitted to this hospital in October, 1949, complaining of shortness of breath and severe coughing spells of seventeen years'

[1] From the Departments of Surgery and Pathology, Veterans Administration Hospital, Coral Gables, Florida.

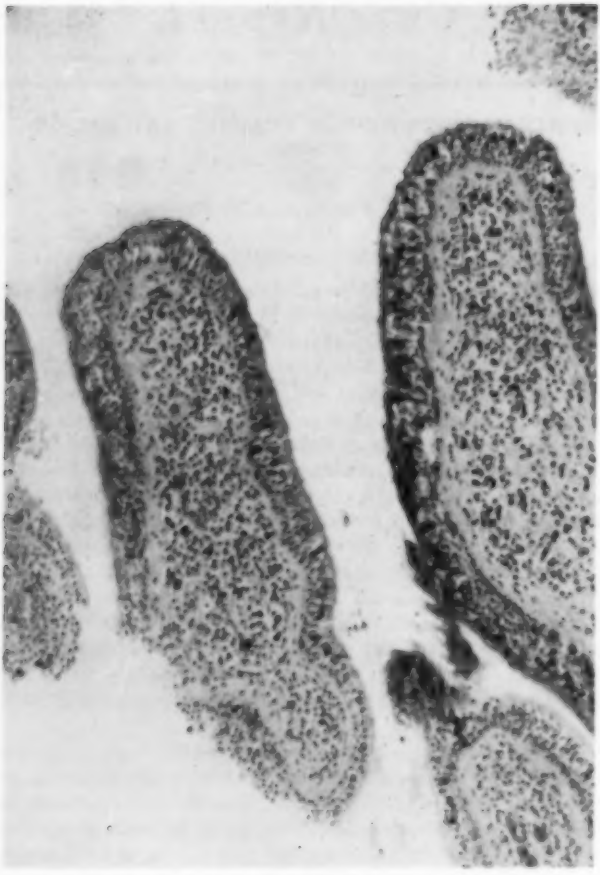


FIG. 1. *Case 1*. Photomicrograph: Fibro-epithelial papillomas of bronchus. (×66)

duration. He was a construction engineer; his first wife had died of pulmonary tuberculosis; he had history of heavy cigarette consumption for most of his adult life. Aside from respiratory symptoms, several episodes of urticaria of unknown etiology were the only illnesses noted in his medical history.

The present illness dated back to 1931, when this patient was hospitalized for treatment of an illness recorded in U.S. Army records as "pneumonia, lobar, right upper lobe, moderately severe, type undetermined." He had been discharged with the process only partially resolved. Thereafter, he developed intermittent dry cough with progressively severe dyspnea. In 1934, 1941, 1944, and 1946, he underwent endoscopic removal of tumors of his right main bronchus. Pathologic findings are not available, but he was informed on each occasion that the tissue removed was "benign." His symptoms disappeared for several months after each operation, only to return and gradually progress in severity until subsequent removal of more tissue was necessary.

Admission examination in 1949 revealed bilateral emphysema. A chest roentgenogram

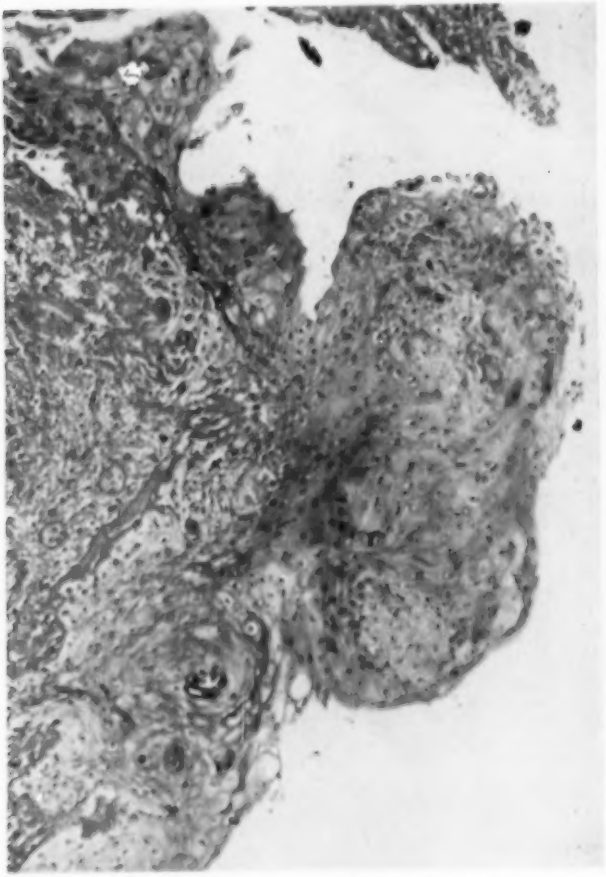


FIG. 2. *Case 1.* Photomicrograph: Papilloma of bronchus with squamous metaplasia.

showed a peritracheal mass in the superior portion of the right hilus. On bronchoscopic examination, a smooth tumor mass was seen projecting into the lumen of the right upper lobe bronchus. This tissue was hard in consistency and was removed with considerable difficulty. Histologic examination showed fragments of bronchial epithelium with underlying scar tissue.

In February, 1952, the patient returned with progressively severe dyspnea and productive cough. Roentgenographic study revealed partial atelectasis of the right lung. On bronchoscopy, a firm, pink wartlike tumor mass was found projecting into the lumen of the right upper lobe bronchus near its origin. Approximately 2 cm. distal to the carina, a similar tumor was seen on the anterolateral wall of the right main bronchus, occluding the lumen at that point. The masses were removed in fragments.

The patient's respiratory difficulty cleared up considerably following the procedure, although the chronic cough persisted, usually after arising in the morning. He has returned at three-month intervals for bronchoscopic examinations; on each occasion moderate re-

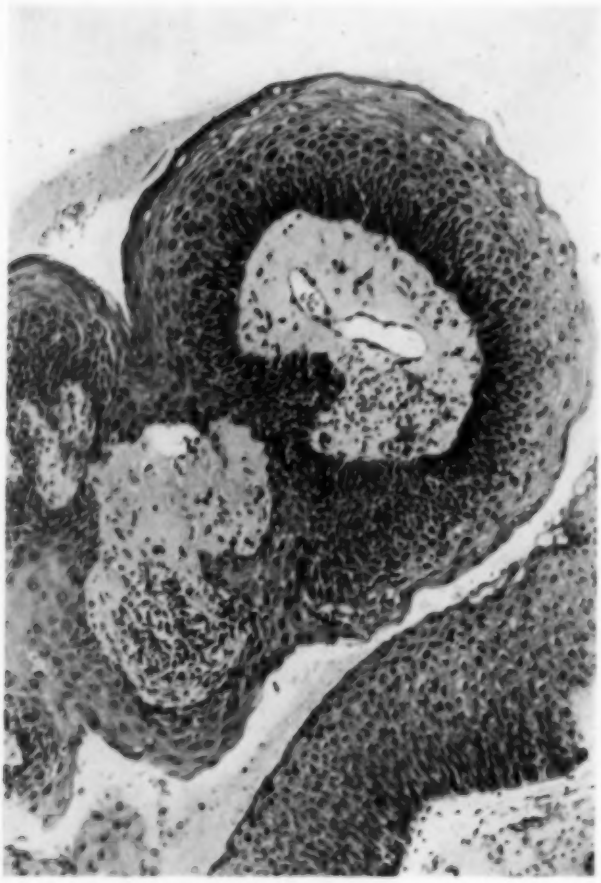


FIG. 3. *Case 2.* Photomicrograph: Squamous papilloma of bronchus. (×65)

spiratory difficulty has been noted, and small papillomatous excrescences have been found, involving proximal portions of the right main bronchus. These have easily been removed, using the endobronchial forceps.

Pathologic studies of tissue removed have consistently showed multiple small polypoid structures with central fibrous and granulation tissue stalks with evidence of subacute and chronic inflammation. The epithelium in most areas consisted of hyperplastic cells of the respiratory type, and rather large scattered foci of metaplastic squamous epithelium were noted. Segments of bronchial wall were removed with many specimens, and have revealed subacute and chronic inflammation with increased vascularity, edema, and small foci of necrosis. No intracellular inclusion bodies suggesting viral infection were found (figures 1 and 2).

Bacteriologic study of removed tissue revealed a mixed flora, predominantly *Streptococcus hemolyticus*, with a lesser growth of *Staphylococcus aureus*. Cultures for viruses and fungi were negative.

Case 2. The patient is a 50-year-old Merchant Marine seaman, who was admitted to this hospital in May, 1954, complaining of shortness of breath and a chronic productive cough for three years. One bout of hemoptysis occurred on the day prior to admission. He had smoked cigarettes for many years but stopped three years before admission; he had smoked a pipe for many years. Hospitalization had been necessary on several previous occasions for respiratory wheezing and cough; a diagnosis of chronic asthma had been made. In 1949, a papilloma of vocal cord was removed, without notable relief of symptoms. There had been no chest pain or weight loss. An old history of malaria and dysentery was recorded.

The physical examination on admission disclosed no abnormalities except an emphysematous thorax with numerous wheezes and rhonchi in all lung fields. Roentgenographic studies showed bilateral emphysema, an inadequately expanded left lung, the lower lobe of which showed focal pneumonitis; there was a mild displacement of the mediastinal structures to the left.

Bronchoscopic examination revealed multiple papillomas involving the tracheal mucous membrane to within 3 cm. of the carina, the distal portion of the left main bronchus, the proximal portion of left upper lobe bronchus, and the basal segments of the left lower lobe bronchus. These masses were removed with a biopsy forceps at several sittings. Bleeding was often profuse, but subsided in a few hours.

Histologic examination of removed tissue revealed papillomas composed of thickened squamous epithelium with narrow vascular and fibrous cores and stalks. Cells were fairly uniform with mild focal pleomorphism, and no epithelial cell inclusions were found (figure 3).

COMMENT

The etiology of bronchial papillomatosis is not entirely known but offers many grounds for speculation. The majority of writers referred to above believed that the tumors were true neoplasms. The multiplicity and wide distribution of the tumors could be attributed to implantation of cells desquamated from a primary focus and aspirated in retrograde fashion to a more distal area. The clinical observation that in most cases the original tumor or tumors were found in the larynx and later appeared in the trachea and bronchi (often after instrumentation of the more proximal masses) lends weight to the implantation theory. The success of Furth (9), who succeeded in 1946 in producing malignant tumors in the lower respiratory tract by installation of tumor cells in the nostrils of mice, is also quite suggestive.

The occurrence of multiple benign or malignant tumors in a continuous epithelial tract, such as urinary and gastrointestinal systems, is seen not infrequently. Although implantation cannot be excluded entirely in such cases, the majority of observers believe that these tumors arise in multicentric fashion in response to a tumorigenic agent which is effective over a wide area.

Case 1 exemplified fibro-epithelial papillomas, the epithelial component of which was normal but hyperplastic bronchial epithelium with focal squamous metaplasia; there was also a marked inflammatory element in the stroma. A similar situation is often seen in chronically inflamed lungs removed at surgery or autopsy. In such cases focal papillary overgrowths of fibro-epithelial tissues are found in smaller bronchi or bronchioles (figure 4).

Case 2 demonstrated multiple squamous papillomas, lesions which may involve skin or various mucous surfaces. There is considerable evidence that such lesions are induced by focal virus infections. Ullman (10) in 1923 and Ishikowa (11) in 1936 showed that cell-free extracts from human laryngeal papillomas produced similar lesions when applied to the surface of oral and vaginal mucous membranes of dogs.

Other viruses cause proliferative changes in cells resulting in papillomata. The best known example is the virus of Shope (12) rabbit papillomatosis. It has been shown that

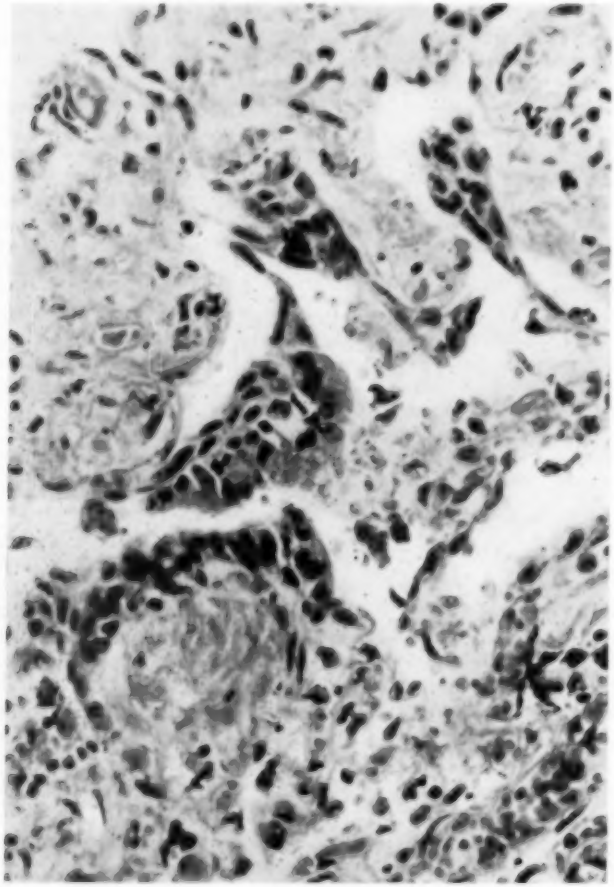


FIG. 4. Photomicrograph: Fibro-epithelial papillomas of smaller bronchi, frequently found in chronic pulmonary disease. (×240)

these viruses induce an orderly proliferation of epidermal cells, followed in some cases, and at long intervals, by malignant transformation. It is now believed that the virus acts in the same manner as other irritating agents, such as tars, arsenic, and sunlight, to cause this malignant transformation. It has been shown that the malignant phase is not directly caused by the virus (13).

The virus theory explains all the manifestations of the disease: the occurrence of multiple papillomata, occasionally following laryngeal papilloma (known to be caused by a virus); the long intervals between the first appearance of the disease and its recurrence; the proliferative changes; and even the very rare malignant transformation. The theory is compatible with the Furth experiment. The virus theory elucidates the multicentric origin of the tumors and furnishes helpful clues to treatment. It suggests that radical

excision is fruitless. (Bronchologists have long recommended endoscopic removal of the tumors.) Perhaps, in the future, a vaccine or some of the newer antimicrobials will be found to be of value.

SUMMARY

Two patients are presented who show the various manifestations of papillomatosis of the lower respiratory tract. The first patient had papilloma in the right main bronchus and the right upper lobe bronchus, with multiple recurrences over a seventeen-year period. The second patient developed a laryngeal papilloma, followed five years later by multiple lesions in the trachea and major bronchi on the left. These lesions were successfully treated by endoscopic removal.

There is a great deal of evidence, both direct and indirect, which suggests that papillary lesions are caused by viral stimulation. Other authors explain the multiplicity of the tumors on the basis of local implantation of desquamated cells.

SUMARIO

Papilomatosis de la Tráquea y de los Bronquios: Presentación de Dos Casos

Preséntase a 2 enfermos que mostraban las varias manifestaciones de la papilomatosis de la porción inferior del aparato respiratorio. El primero tenía papiloma en el bronquio del lóbulo superior derecho, con múltiples recurrencias durante un período de diecisiete años. El segundo enfermo manifestó un papiloma laríngeo, seguido cinco años después de lesiones múltiples en la tráquea y los bronquios mayores a la izquierda. Esas lesiones fueron tratadas con éxito mediante la extirpación endoscópica.

Hay muchos datos, tanto directos como indirectos, que sugieren que esas lesiones papilares son ocasionadas por excitación viral. Explican otros autores la multiplicidad de los tumores a base del implante local de células descamadas.

RESUME

Papillomatose de la trachée et des bronches: Rapport sur deux cas

Les observations de deux malades présentant les diverses manifestations de la papillomatose des voies respiratoires inférieures sont rapportées. Le premier patient avait un papillome dans la bronche souche droite et dans la bronche supérieure droite, la récurrence des lésions s'était multipliée au cours d'une période de dix-sept ans. Le second patient avait eu un papillome du larynx auquel avait succédé de multiples lésions au niveau de la trachée et des grosses bronches gauches. Ces lésions furent traitées avec succès par la résection endoscopique. De nombreuses preuves, à la fois directes et indirectes, permettent de penser que ces lésions papillaires sont provoquées par un virus. D'autres auteurs expliquent la multiplicité de ces tumeurs sur la base d'une implantation locale de cellules desquamées.

REFERENCES

(1) Syme, W. S.: Three bronchoscopic cases, J. Laryng. & Otol., 1927, *42*, 751.

(2) Zamora, A. M.: Two benign growths removed by bronchoscopy, J. Laryng. & Otol., 1931, *46*, 829.

(3) Orton, H. B.: Papilloma of the bronchus, Ann. Otol., Rhin. & Laryng., 1932, *41*, 933.

(4) Jackson, C., and Jackson, C. L.: Benign tumors of the trachea and bronchi, with special reference to tumor-like formations of inflammatory origin, J. A. M. A., 1932, *99*, 1747.

(5) Pollak, B. S., Cohen, S., and Gnassi, A. M.: Inflammatory bronchial tumors, Arch. Otolaryng., 1938, *27*, 426.

(6) Patterson, E. J.: Multiple papilloma of larynx, trachea and left bronchus in a child, Ann. Otol., Rhin. & Laryng., 1939, *48*, 1080.

(7) Buffmire, D. K., Clagett, O. T., and McDonald, J. R.: Papillomas of the larynx, trachea and bronchi: Report of case, Proc. Staff Meet., Mayo Clin., 1950, *25*, 595.

(8) Liebow, A. A.: Tumors of the Lower Respiratory Tract, *Atlas of Tumor Pathology*, Sec. V, Fasc. 17, Armed Forces Institute of Pathology (1952).

(9) Furth, J.: Experiments on the spread of neoplastic cells through the respiratory passages, Am. J. Path., 1946, *22*, 1101.

(10) Ullman, E. V.: On the aetiology of the laryngeal papilloma, Acta oto-laryng., 1923, *5*, 317.

(11) Ishikawa, K.: Klinische und experimentelle Untersuchungen über die Entstehungsursachen der Papillome, Fukuoka Acta. med. (abstract section), 1936, *29*, 87.

(12) Shope, R. E.: Infectious papillomatosis of rabbits, J. Exper. Med., 1933, *58*, 607.

(13) Kidd, J. G.: The Pathogenesis and Pathology of Viral Diseases, Columbia University Press, New York, 1950, p. 161.

TOTAL TUBERCULOUS CALCIFICATION OF A KIDNEY AND URETER[1]

LEWIS G. JACOBS

(Received for publication July 12, 1954)

Although renal tuberculosis is a well-known cause of renal calcification, the development of total calcification during observation has rarely been reported. Accordingly, the present case of a patient with renal tuberculosis who developed a total calcification of the kidney and ureter during the course of his illness is deemed worthy of a report. The case has three points of interest: the development of calcification, from none to total in four and one-half years; the absence of any clear evidence of urinary tract obstruction; and the presence of a complicating duodenal ulcer without evidence of pyloric obstruction.

The causes of renal calcification may be divided into three groups (1, 2):

Metastatic calcification accompanying bone disease with hypercalcemia

Calcifications associated with a lesion causing a decrease of solubility of calcium salts in the blood and tissue fluids, or causing impairment of calcium excretion

Calcification in damaged or necrotic renal tissue

Among causes in the second group have been upper gastrointestinal tract obstruction and prolonged vomiting; the mechanism by which these cause calcification is not entirely clear. The characteristic calcification is in the tubular epithelium. A toxic nephrosis has been described in some cases, while in others calcification has been ascribed to secondary renal alkalosis. The injection of neutral, but not of acid, chloride salts exerts a protective action in cases of this type. To the writer's knowledge, no such calcification has been reported as the result of an unobstructed ulcer, nor have the patients in the reported cases shown total calcification. The possibility that the persistent use of antacid medication could sufficiently disturb the acid-base balance to produce such renal calcification or to seriously contribute to its production seems unlikely.

Infectious calcifications belong in the third group. Braasch and Olson (3) classify tuberculous renal calcifications as follows: multiple small scattered areas, either streaky or punctate; single or few isolated, circumscribed areas of irregular outline and density; a large irregular or diffuse area involving most or all of the kidney. These classes are obviously not sharply demarcated. The first and third are considered pathognomonic of tuberculosis (4), but the second may have one of the other types of cause noted above.

The present case falls into the third class, and such cases of total unilateral calcification are rather uncommon. Crenshaw (5) reported that 131 (approximately 7 per cent) of 1,817 cases of renal tuberculosis seen at the Mayo Clinic showed some degree of calcification, but only 41 of these (approximately 2 per cent) showed complete calcification. Both Braasch (6) and Crenshaw express the opinion that ureteral obstruction is a major factor in the development of total calcification. Symptoms may be slight or absent. The treatment, indicated only in symptomatic cases, is usually a ureteronephrectomy.

CASE REPORT

A white male patient was first seen on March 29, 1948, at the age of forty-nine years for treatment of a duodenal ulcer which had first been diagnosed in 1938. He had been treated for tuberculosis of the right hip in 1926, for "heart disease" in 1944, and (at the U. S. Marine

[1] From the Veterans Administration Hospital, Oakland, California.

FIG. 1. KUB on April 5, 1948. Stones seen on right; left kidney, normal.

Hospital in San Francisco) for renal and vesical tuberculosis in 1947. In February, 1947, cultures of both the bladder and left renal (catheterized) urine were positive for tubercle bacilli. An intravenous pyelogram in May, 1947, was reported as showing poor visualization of the left kidney with gas obscuring the upper calyces of both kidneys. The inferior calyces were not definitely abnormal.

Examination showed, among other abnormalities, enlargement of the left seminal vesicle. There were signs and symptoms of duodenal ulcer. Roentgenographic study confirmed this diagnosis, but no pyloric obstruction was present. The patient's cardiovascular system was normal on clinical examination; the blood pressure was 110 systolic, 80 diastolic in mm. of mercury; and the electrocardiogram was normal.

An intravenous pyelogram on April 5 showed that the right kidney contained multiple calculi in the pelvis, and poor excretion on the left, but no definite abnormality of outline. The plain film of the abdomen (KUB) made at this time is shown in figure 1.

FIG. 2. KUB on October 4, 1954. Total calcification of left kidney and ureter. Stones persist on right.

A roentgenogram of the pelvis showed loss of the head and neck of the right femur. The laboratory tests gave a blood urea nitrogen of 17 mg. per 100 ml., a negative test for syphilis (Kahn), and a count of 4.5 million erythrocytes per cu. mm., 14 gm. hemoglobin, 7,100 leukocytes per cu. mm., 65 per cent neutrophils, 32 per cent lymphocytes, 1 per cent monocytes, and 2 per cent eosinophils. As the patient refused further urological examination, he was discharged improved, after treatment of his duodenal ulcer with a modified Sippy regimen. He was instructed to take one-half grain of phenobarbital and atropine (1/100 grain) daily, and Amphogel® (suspension of aluminum hydroxide) as needed.

The patient re-entered the hospital on October 1, 1952, complaining of epigastric pain similar in character to his previous pain, but now constantly present for two weeks. He also had nocturia and terminal dysuria. Swelling of the left epididymis was now painful, and the right was also swollen. Pus was discharging from multiple scrotal sinuses. The blood pressure was 112 systolic, 80 diastolic in mm. of mercury. A chest roentgenogram was normal. A KUB on October 4 showed total calcification of the left kidney and ureter (figure

2), and the cystoscopic report on this day states "The right and left ureteral orifices were wide and gaping." Laboratory findings were: blood urea nitrogen, 200 mg. per 100 ml.; 2.4 million erythrocytes per cu. mm., 6.4 gm. hemoglobin, 10,700 leukocytes, 87 per cent neutrophils, 13 per cent lymphocytes. Urine cultures were negative for tubercle bacilli. Treatment for the ulcer, neglected by the patient, was reinstituted as before. The patient experienced increasing nausea and vomiting, and died in uremia on October 13. Permission for autopsy was refused.

SUMMARY

This article reports the case of a man who developed total calcification of an unobstructed tuberculous kidney and ureter during a four and one-half year period of observation.

SUMARIO

Calcificación Tuberculosa Total de un Riñón y su Uréter

Preséntase el caso de un sujeto que manifestó calcificación total de un riñón y uréter tuberculosos inocluídos durante un período de cuatro y medio años de observación.

RESUME

Calcification tuberculeuse complète d'un rein et de son uretère

Cet article rapporte le cas d'un homme ayant présenté une calcification complète d'un rein tuberculeux perméable et de son uretère au cours d'une période d'observation de quatre ans et demi.

REFERENCES

(1) Ewell, G. H.: Calcification of the kidney and ureter, Urol. & Cutaneous Rev., 1941; *45*, 150.

(2) Martz, H.: Renal calcification accompanying pyloric and high intestinal obstruction, Arch. Int. Med., 1940, *65*, 375.

(3) Braasch, W. F., and Olson, E. A.: Roentgenologic diagnosis in renal tuberculosis, Surg., Gynec., & Obst., 1919, *28*, 555.

(4) Goldstein, A. E., and Abeshouse, B. S.: Calcification and ossification of the kidney: Review of the literature and report of cases, Radiology, 1938, *30*, 544; 667. (*This includes an excellent bibliography.*)

(5) Crenshaw, J. L.: Renal tuberculosis with calcification, J. Urol., 1930, *23*, 515.

(6) Braasch, W. F.: Tuberculosis of the kidney (Transactions of the Chicago Urological Society), J. Urol., 1930, *23*, 669.

Editorial

Implications of the Phenomenon of Open Cavity Healing for the Chemotherapy of Pulmonary Tuberculosis

Several reports of so-called healing of tuberculous cavities in the open, air-filled state have recently appeared[1-7]. Although some authorities are of the opinion that such "cysts" are not immediate derivatives of true tuberculous cavities, in our experience[8] there is justification for believing that in some cases they are. It would appear that there has been a recent increase in the frequency of this phenomenon. This we attribute to the use of the various antimicrobial agents which are now available. It is our belief that the time has come for important modifications in the chemotherapeutic approach to tuberculosis. We believe that it is possible to formulate a better defined attack on tubercle bacilli multiplying within open cavities. In our opinion the primary aim of chemotherapy is to render the excreta of patients bacteriologically negative for tubercle bacilli. While there are many problems related to microbial resistance which have both theoretical and practical importance, perhaps concern for these problems has hypnotized much of the recent thinking devoted to the chemotherapy of this disease. Our primary aim is the destruction of tubercle bacilli. Therefore, the purpose of this editorial is to offer certain concepts which may be useful in explaining the mode of action of the various antimicrobial agents in effecting sterilization of the open tuberculous cavity in some patients.

In presenting this material, we are well aware of the fact that it is highly selected, supports our arguments, and ignores material which we deem to be irrelevant. This is, after all, a highly controversial subject. To our knowledge, no similar effort to explain the phenomenon of open cavity healing has as yet been published. We hope that the basic experimental data which we are integrating here will provide an adequate foundation for understanding the dynamics of this process.

Both streptomycin and isoniazid *in vitro* are actively bactericidal for human and bovine strains of *Mycobacterium tuberculosis* only when the organisms are multiplying[9, 10]. Probably, neither streptomycin nor isoniazid has an appreciable effect on tubercle bacilli in closed necrotic lesions, in which anaerobic conditions

[1] Purriel, P., *et al.*: Tórax, 1953, *2*, 115.

[2] Bernard, E., and Carrand, J.: Rev. de la tuberc., 1953, *17*, 1021.

[3] Galy, P., *et al.*: Rev. de la tuberc., 1953, *17*, 1037.

[4] Pruvost, P., *et al.*: Rev. de la tuberc., 1953, *17*, 1046.

[5] Simonin, *et al.*: Rev. de la tuberc., 1953, *17*, 1051.

[6] Bernou, A.: Rev. de la tuberc., 1953, *17*, 1055.

[7] Altman, V., and Ornstein, G. G.: Quart. Bull. Sea View Hosp., 1954, *15*, 1.

[8] Russell, W. F., Jr., *et al.*: Healing of the open cavity in pulmonary tuberculosis with streptomycin and isonizaid therapy (to be published).

[9] Middlebrook, G., and Yegian, D.: Am. Rev. Tuberc., 1946, *54*, 533.

[10] Schaefer, W. B.: Am. Rev. Tuberc., 1954, *69*, 125.

are thought to prevail. Studies utilizing living macrophages have demonstrated that para-aminosalicylic acid is effective against only extracellular tubercle bacilli; streptomycin is effective against extracellular organisms and is relatively ineffective against intracellular bacteria at drug concentrations usually attained *in vivo*; and isoniazid is almost equally effective against both extra- and intracellular tubercle bacilli[11-13]. In pulmonary lesions, tubercle bacilli multiply extracellularly in great numbers, mainly in the softening necrotic material in open cavities[14]. Therefore, on the basis of the intracellular versus extracellular location of the parasites, streptomycin and para-aminosalicylic acid should be active against tubercle bacilli multiplying in open cavities. In addition, penetration of streptomycin into the cavity has been demonstrated by chemical methods[15]. Isoniazid has also been proved by radioactive isotope studies to penetrate rapidly into both cavitary and caseous nodular lesions[16]. Therefore, in theory, multiplying tubercle bacilli within the cavity should be susceptible to the sterilizing action of both streptomycin and isoniazid.

Florey[17] suggested that the relative avascularity of the tuberculous lesion might prevent attainment of therapeutic concentrations of antimicrobial agents within the cavity. However, at the time when the presence of the drugs is most necessary, the open tuberculous cavity is lined with well-vascularized granulation tissue[18], even though the larger pericavitary arteries may be obstructed. Therefore, the thickness of the wall and its vascularization probably are not obstacles to the achievement of adequate concentrations of these chemotherapeutic agents within the lumen of the cavity.

When patients are treated with isoniazid alone in adequate dosage, the reduction in numbers of drug-susceptible bacilli in the sputum, together with the usual prompt appearance of drug-resistant organisms, is thought to be evidence that this agent is active within the cavity. The same argument theoretically would appear to apply to streptomycin when employed alone. Twelve of ninety-six patients whose original treatment was 1.0 gm. of streptomycin every third day and 8 mg. of isoniazid per kg. daily for a period of six to eighteen months continued to discharge tubercle bacilli in the sputum for at least six months. Organisms highly resistant to isoniazid were present in the sputum of each of these patients before bacteriologic conversion (post-surgical) occurred. No significant numbers of streptomycin-resistant (to 10 γ of streptomycin per ml. of medium) tubercle bacilli appeared. The rare appearance of streptomycin-

[11] Suter, E.: Am. Rev. Tubere., 1952, *65*, 775.

[12] Mackaness, G. B.: J. Path. & Bact., 1952, *64*, 429.

[13] Mackaness, G. B., and Smith, N.: Am. Rev. Tubere., 1953, *67*, 322.

[14] Canetti, G.: Le Bacille de Koch dans la Lesion Tuberculeuse du poumon, Edition Médicales Flammarion, Paris, 1946.

[15] Steenken, W., Jr., *et al.*: Am. Rev. Tubere., 1947, *56*, 403.

[16] Barclay, W. R., *et al.*: J. A. M. A., 1953, *151*, 1384.

[17] Florey, H.: Proc. Roy. Soc. Med., 1952, *45*, 71.

[18] Davis, B. D., and McDermott, W.: Principles of chemotherapy, in *Bacterial and Mycotic Infections of Man*, ed. 2, edited by R. Dubos, J. B. Lippincott Co., Philadelphia, 1952.

resistant organisms in the sputum after large numbers of isoniazid-resistant organisms have appeared strongly suggests that streptomycin on this low dosage regimen was not biologically active against that part of the bacterial population within the cavity which *was* exposed to isoniazid.

Granted that streptomycin is chemically present within the cavity, why is it not biologically active? It has been shown that desoxyribonucleic acid is present in the contents of tuberculous cavities. Cohn, Kovitz, and Middlebrook[19] have demonstrated that desoxyribonucleic acid antagonizes streptomycin *in vitro*. Thus, it would appear to be a possibility that streptomycin would be antagonized by desoxyribonucleic acid as it diffuses into the open cavity in clinical cases. This implies that combined therapy with streptomycin and isoniazid, when applied to open cavities, might well be, in most cases, equivalent to treatment with isoniazid alone.

The necessity for employing more than one drug at the same time seems to be well established. Yegian and Vanderlinde[20], in an extensive review, concluded that resistance is not "induced" by the action of the antimicrobial agent on susceptible organisms. Resistant strains of mycobacteria appear principally as a consequence of selective action by the antimicrobial agent. The organisms which survive exposure to the drug are mainly those spontaneously appearing mutants which are by nature resistant. Each wild race of tubercle bacilli studied in our laboratories has been found to yield an individual, but more or less constant, incidence of spontaneous isoniazid-resistant mutants. Similarly, strains of tubercle bacilli exhibit spontaneous mutation to streptomycin-resistant forms at individual, but constant, rates[21-23]. Streptomycin-resistant strains of tubercle bacilli are fully susceptible to isoniazid, and vice versa. The rate of spontaneous mutation of tubercle bacilli to forms that are highly resistant to both isoniazid and streptomycin at the same time is extremely low[21, 23, 24]. It should be emphasized that this very low rate of spontaneous mutation of tubercle bacilli to forms that are resistant to both isoniazid and streptomycin occurs only when susceptible organisms are exposed to these agents simultaneously and, clinically, early in the period of therapy. If both drugs reach all multiplying bacilli within a short period of time, spontaneously appearing mutants which might be resistant to one drug should sooner or later be sterilized by the other. Probably the bacterial population within the cavity is seldom so large that organisms naturally resistant to both streptomycin and isoniazid are numerically significant at the beginning of treatment[24]. Therefore, if the tubercle bacilli in the cavity are exposed to *effective* concentrations of both drugs together and for the first time, the total population of bacilli in the cavity *should be sterilized*.

While this desired result may have occurred in some of our ninety-six pa-

[19] Cohn, M. L., *et al.*: To be published.
[20] Yegian, D., and Vanderlinde, R. J.: Am. Rev. Tubere., 1950, *61*, 483.
[21] Middlebrook, G.: Am. Rev. Tubere., 1952, *65*, 765.
[22] Jensen, K. A.: Acta tubere. Scandinav., Supplement XXI, 1949, p. 42.
[23] Singh, B., and Mitchison, D. A.: Brit. M. J., 1954, No. 4854, 130.
[24] Mitchison, D. A.: Brit. M. Bull., 1954, *10*, 115.

tients who had not had any previous treatment, it did not occur in the twelve who remained infectious for at least six months. Therefore, we believe that the appearance of isoniazid-resistant forms in these cases is evidence that streptomycin was not biologically active within the cavity. The fact that no significant numbers of streptomycin-resistant organisms appeared as therapy was continued is further evidence that streptomycin was not active within the lumen of the cavity. The combined weight provided by these two facts constitutes compelling evidence that this is true.

And yet, formerly when streptomycin was used as the sole therapeutic agent, organisms resistant to it routinely appeared in the sputum. In those cases in which very high dosage regimens were employed, it is possible that enough streptomycin diffused into the cavity to provide an effective excess of biologically active streptomycin. On the other hand, streptomycin-resistant organisms also were recovered from the sputum of patients on lower dosage schedules. We believe that such streptomycin-resistant strains probably do not originate from the multiplying bacterial population in the caseous contents of the cavity. The binding action of desoxyribonucleic acid which renders streptomycin inactive might prevent the attainment of effective concentrations of the drug in this location. Therefore, resistant organisms that appear in the sputum on lower dosage regimens should be selected at a site in the cavity which is relatively remote from the "pyogenic membrane," that is, from free desoxyribonucleic acid.

Canetti[14] clearly demonstrated that, in the untreated tuberculous cavity, the majority of the bacilli were located superficially in the "pyogenic membrane." However, most significantly, he noted that a few intra- and extracellular organisms were located in the peripheral granulation tissue of the wall. Organisms at this site are relatively remote from the autolyzing cells, from which desoxyribonucleic acid presumably is derived. Therefore, as the streptomycin diffuses out from the capillaries in the granulation tissue, it might be biologically active against those organisms that are present in this special location. It is possible that these peripheral extracellular bacteria could be the source of the streptomycin-resistant mutants which eventually are shed into the necrotic contents of the cavity. The more centrally located organisms in the caseous material may remain drug-susceptible because the streptomycin does not reach them in effective concentration. Sooner or later significant numbers of resistant bacilli would multiply in the cavity, and, therefore, be found in the sputum. Indeed, in many cases they may ultimately constitute a significant percentage of the organisms multiplying in the contents of the cavity. Critical analysis of techniques of tests for drug resistance indicates that only rarely may all bacterial cells excreted from the cavities of patients under streptomycin therapy be resistant.[24]

Para-aminosalicylic acid is blocked in its action by the presence of para-aminobenzoic acid[25]. This substance is known to be present in necrotic debris,

[24] Middlebrook, G.: Am. Rev. Tuberc., 1954, *70*, 922.

[25] Youmans, G. P., *et al.*: J. Bact., 1947, *54*, 409.

and presumably is present in the "pyogenic membrane" in cavities. It would appear, then, that when streptomycin and para-aminosalicylic acid were used in combination, effective combined drug therapy was achieved *in a limited sense*. This was due to the fact, we believe, that both drugs were unable to exert antibacterial effects on the tubercle bacilli multiplying in the lumen of the cavity. In all likelihood they acted mainly on those organisms lying in the peripheral granulation tissue. At this site they were able to act together because the substances which could block their action were relatively remote and were being excreted through the draining bronchus.

If this thesis is correct, then the failure of streptomycin-resistant mutants to appear in the sputum of our previously untreated patients on a regimen of streptomycin and isoniazid can be explained. The extracellular organisms which are located within the peripheral granulation tissue probably are subjected to the simultaneous action of both drugs with the result that they no longer multiply, and many are sterilized. The intracellular organisms and those multiplying within the "pyogenic membrane" may be subjected only to the action of isoniazid. Isoniazid-resistant forms, therefore, are selected. The one agent which is known to antagonize streptomycin and to appear in the contents of the cavity, desoxyribonucleic acid, may well be the main obstacle in preventing "sterilization of the open cavity." Although other products of autolyzing tissue may also block the antibacterial action of streptomycin, they have not been identified. In any event, their activity in this respect would not alter the hypothesis presented here.

Elimination of the caseous material from the cavity would not only tend to reduce the blocking action of desoxyribonucleic acid but also would deprive the bacilli of their "culture medium." In the natural evolution of the cavity, caseous necrotic tissue softens spontaneously, but usually slowly and incompletely. In a study of our patients who were treated solely with isoniazid, early and extensive liquefaction of caseous pulmonary tissue was an outstanding host reaction.[27] The term *chemical caseotomy* was coined by Purriel and his associates[1] because they were impressed by the unique, possibly total, elimination of caseous material from the cavities of isoniazid-treated patients. Curci[28] implanted caseous material from tuberculous guinea pigs subcutaneously into hypersensitive guinea pigs. In animals which were then treated with isoniazid, liquefaction and absorption were more rapid than in the controls. On the contrary, in animals treated with streptomycin alone, liquefaction was inhibited and encapsulation occurred. It seems reasonable, at least in some instances, to assume that isoniazid promotes the liquefaction of caseous tissue.

The fact that more cases of open cavity healing are being seen following the use of various regimens of combined drug therapy, especially when isoniazid is employed, suggests, indeed, that in certain very special cases the drugs are completely effective in the sense indicated here. Actually, in current practice, the real potential of streptomycin and isoniazid in combination is not realized

[27] Denst, J.: Am. Rev. Tuberc., 1953, *68*, 144.
[28] Curci, G.: Arch. di tisiol., 1953, *8*, 206.

frequently enough. Among a great variety of factors which may be responsible, there are several that suggest themselves as having great importance. Caseous material may fail to soften. Premature closure of the bronchus may hamper the elimination of the softened caseous necrotic tissue. Delay in mechanical elimination of caseous material might continuously block one of the drugs from activity within the cavity, and so allow organisms resistant to the other to be selected. Irreversible fibrotic pericavitary lesions might interfere with the mechanics of emptying. In any case the consequence of these obstacles is the retention of caseous material in the cavity.

It appears logical to propose a different approach from the usual therapeutic regimens now employed when one is dealing with open, cavitary pulmonary tuberculosis which has not previously been treated. Some preliminary measures may profitably be used which would tend to reduce the numbers of tubercle bacilli multiplying in the cavity to a minimum. In addition, efforts should be directed at preventing the accumulation and hastening the elimination of caseous necrotic tissue from the cavity. Finally, when the open lesions have been prepared for healing, treatment with our most effective drugs should be started. In view of the obvious importance which we attach to sterilizing as many of the tubercle bacilli as possible, as early as possible, no apology need be tendered for our emphasis on the early phase of therapy. We believe that the use of high doses of the various antimicrobial agents when the cavity is specially prepared offers hitherto unsuspected opportunities in the therapy of cavitary pulmonary tuberculosis.

This approach seems all the more important in view of the fact that it is the persistently open cavity containing caseous necrotic material and, too often, drug-resistant tubercle bacilli which is the most outstanding stumbling block today in the chemotherapy of tuberculosis.

W. F. Russell, Jr.
S. H. Dressler
G. Middlebrook
J. Denst
Denver, Colorado

Notes

DRUG SUSCEPTIBILITIES OF M. TUBERCULOSIS ON CHARCOAL AGAR MEDIUM

Recently a charcoal agar medium has been introduced for the cultivation of tubercle bacilli[1]. Growth of mycobacteria on this medium compares favorably with that obtained on egg-potato-glycerol and on oleic-acid–albumin media now in common use. The charcoal agar medium, in contrast to media currently employed, is economical and is easy to prepare.

As the charcoal agar medium may prove satisfactory for clinical use, its applicability to drug-susceptibility tests is of some interest. The present communication reports experiments dealing with this point.

In the initial studies, a comparison was made of the drug susceptibility of two laboratory strains of tubercle bacilli to streptomycin, PAS, and isoniazid on charcoal agar[1] and on oleic-acid–albumin media[2]. The mycobacteria used were H37Rv (human virulent) and Vallée (bovine virulent), neither of which had ever been exposed to antimycobacterial drugs.

The data in table 1 reveal that the concentration of streptomycin and of PAS required for inhibition of growth was approximately the same in these two media.

However, when isoniazid was tested, there was a marked difference in the concentration required for inhibition of growth on charcoal agar as compared to oleic-acid–albumin agar. With both human and bovine strains of mycobacteria, the inhibitory concentration of isoniazid in charcoal agar was approximately twenty times that in oleic-acid–albumin medium. Thus it appeared that isoniazid was antagonized or inactivated in some fashion in the charcoal medium.

Additional experiments were done to observe the effect of isoniazid on the growth of resistant strains of tubercle bacilli on charcoal agar medium. The results are presented in table 2.

At the outset, it should be pointed out that the quantity of growth of the isoniazid-resistant tubercle bacilli in a given period of time was markedly less than that of isoniazid-susceptible mycobacteria, whether they were cultivated on charcoal agar or on oleic-acid–albumin medium. As is shown in table 2, with the highly isoniazid-resistant strains RA and RB, the end points on the two media were approximately the same. However, in the case of the mildly resistant strain RC, the determinations were markedly different. On charcoal medium, the susceptible strain H37Rv (*see* table 1) and the slightly resistant strain RC (*see* table 2) were inhibited by the same isoniazid concentration, while on oleic-acid–albumin medium ten times more isoniazid was necessary to inhibit RC than H37Rv. Thus, the charcoal medium, in the form employed, could not be used to differentiate between susceptible bacilli and strains resistant to low concentrations of isoniazid.

The mechanism of the inactivation of isoniazid in the charcoal agar medium was next considered. As charcoal is known to adsorb a variety of substances, experiments were performed to determine whether or not adsorption of isoniazid took place. Quantities of charcoal similar to those present in the culture medium (0.1 per cent) were suspended in aqueous solutions of ascending concentrations of isoniazid. After standing at room temperature for fifteen minutes, the charcoal was separated by filtration through paper, and the concentration of isoniazid was then determined in the filtrates, utilizing a color

[1] Hirsch, J. G.: Am. Rev. Tubere., 1954, *70*, 955.

[2] Dubos, R. J., and Middlebrook, G.: Am. Rev. Tubere., 1947, *56*, 334

TABLE 1

SUSCEPTIBILITY OF TUBERCLE BACILLI TO VARIOUS DRUGS ON CHARCOAL AGAR AND ON OLEIC-ACID-ALBUMIN AGAR

Antimycobacterial Drug		Growth of Tubercle Bacilli			
		H37Rv (Human Virulent)		Vallée (Bovine Virulent)	
		Charcoal Agar	Oleic-Acid-Albumin Agar	Charcoal Agar	Oleic-Acid-Albumin Agar
	γ per ml.				
Streptomycin*	2.00	0†	0†	0†	0†
Streptomycin*	1.00	±	0	0	0
Streptomycin*	0.50	++++	+++	++	±
Streptomycin*	0.25	++++	++++	++	++++
Streptomycin*	0.12	++++	++++	++++	++++
Streptomycin*	0.00	++++	++++	++++	++++
PAS‡	2.00	0	0	+	+
PAS‡	1.00	0	0	++	++
PAS‡	0.50	+	0	++++	++++
PAS‡	0.25	++	+	++++	++++
PAS‡	0.12	++++	++++	++++	++++
PAS‡	0.00	++++	++++	++++	++++
Isoniazid‡	2.500	+	0	0	0
Isoniazid‡	1.000	+++	0	0	0
Isoniazid‡	0.500	++++	0	++++	0
Isoniazid‡	0.200	++++	0	++++	0
Isoniazid‡	0.100	++++	++	++++	++
Isoniazid‡	0.050	++++	+++	++++	+++
Isoniazid‡	0.025	++++	++++	++++	++++
Isoniazid‡	0.000	++++	++++	++++	++++

* Added aseptically to sterilized medium.

† Growth after incubation for 19 to 21 days estimated by visual examination and graded from 0 (no growth) to ++++ (growth equal to that on the same medium containing no antimicrobial drug). The inoculum was 0.025 ml. of a 1:1,000 dilution of a fully grown dispersed culture in liquid medium.

‡ Autoclaved with the medium.

reaction for this determination.[3] No isoniazid was detectable[4] in the filtrates from solutions originally containing less than 10 γ of isoniazid per ml. Filtrates of the more concentrated isoniazid solutions contained demonstrable isoniazid, but in quantities more than tenfold less than the corresponding controls to which no charcoal had been added. Thus the reduced antimycobacterial activity of isoniazid in charcoal agar medium appeared, indeed, to be due to inactivation of isoniazid by charcoal. The limit in the amount of isoniazid inactivated by the quantity of charcoal used suggested that the inactivation process might be an adsorption, with saturation of the charcoal at high concentrations of isoniazid.

[3] SHORT, E. I.: Lancet, 1954, *1*, 656.

[4] The color test used was not capable of detecting concentrations of isoniazid less than 1 γ per ml.

TABLE 2

COMPARISON OF ISONIAZID SUSCEPTIBILITY OF RESISTANT STRAINS OF M. TUBERCULOSIS AS DETERMINED ON CHARCOAL AGAR AND ON OLEIC-ACID–ALBUMIN AGAR

Isoniazid Concentration	Growth of Tubercle Bacilli					
	RA*		RB*		RC*	
	Charcoal Agar	Oleic Acid–Albumin Agar	Charcoal Agar	Oleic Acid–Albumin Agar	Charcoal Agar	Oleic Acid–Albumin Agar
γ per ml.						
333.00	0†	0†	0†	0†	0†	0†
100.00	+	++	0	0	0	0
33.00	+++	++++	++++	0	0	0
10.00	++++	++++	++++	++++	0	0
3.00	++++	++++	++++	++++	++	0
1.00	++++	++++	++++	++++	++++	0
0.30	++++	++++	++++	++++	++++	+++
0.10	++++	++++	++++	++++	++++	++++
0.00	++++	++++	++++	++++	++++	++++

* Isoniazid-resistant strains of *M. tuberculosis* originally isolated from patients at The New York Hospital. These strains were obtained through the courtesy of Dr. Ralph Tompsett. Inoculum was 0.025 ml. of a 1:1,000 dilution of fully grown dispersed cultures in liquid medium.

† Growth after 19 days estimated by visual examination and graded from 0 (no growth) to ++++ (growth equal to that on the same medium containing no antimicrobial drug).

Attempts were made to saturate the charcoal with isoniazid prior to incorporation in the medium. When such charcoal was used in culture medium, however, the growth of tubercle bacilli was in all cases inhibited, even when no isoniazid was added.

One other approach was used in efforts to modify the charcoal agar medium so as to render it more satisfactory for isoniazid-susceptibility determinations. Other substances were sought which might compete with isoniazid for adsorption onto the charcoal. Such a substance, if relatively nontoxic to the growth of mycobacteria, might be added to the medium in high concentrations and might thus largely displace isoniazid from the charcoal. Organic bases and compounds similar in molecular structure to isoniazid were studied. Of these substances, which included niacin, arginine, putrescine, phenyl hydrazine, hydrazine hydrochloride, histamine, spermine, and semicarbazide, only semicarbazide was found to be adsorbed to charcoal in significant amounts. These observations thus suggested that the carbonyl-hydrazine (R—CO—NH—NH_2) grouping might be the portion of the isoniazid molecule responsible for its adsorption to activated charcoal.

In view of the above findings, isoniazid-susceptibility tests were done in charcoal agar media to which semicarbazide had been added. The results are presented in table 3. As is shown in this table, the addition of semicarbazide did enhance considerably the antibacterial activity of isoniazid. In charcoal agar containing 100 γ of semicarbazide hydrochloride per ml., 0.25 γ of isoniazid per ml. brought about significant inhibition of growth of H37Rv (susceptible strain), whereas in plain charcoal agar an isoniazid concentration approximately ten times higher was required to achieve the same effect. It should be pointed out, however, that the inclusion of semicarbazide hydrochloride at 100 γ per ml. did not completely eliminate the inactivation of isoniazid in charcoal agar, since the

TABLE 3

THE EFFECT OF SEMICARBAZIDE ON ISONIAZID-SUSCEPTIBILITY DETERMINATIONS ON CHARCOAL AGAR MEDIUM

Concentration of Isoniazid	Growth of Tubercle Bacilli							
	H37Rv (Human, Isoniazid-Susceptible)				RD (Human, Isoniazid-Resistant)*			
	Oleic-Acid-Albumin Medium	Charcoal Agar Medium Semicarbazide-HCl 100 γ per ml.	Charcoal Agar Medium Semicarbazide-HCl 50 γ per ml	Charcoal Agar Medium No Semicarbazide	Oleic-Acid-Albumin Medium	Charcoal Agar Medium Semicarbazide-HCl 100 γ per ml.	Charcoal Agar Medium Semicarbazide-HCl 50 γ per ml.	Charcoal Agar Medium No Semicarbazide
γ per ml.								
8.00	0†	0†	0†	+†	0†	0†	+†	++†
4.00	0	0	0	+	0	0	++	++++
2.00	0	0	+	+++	++	++	++++	++++
1.00	0	0	+	++++	++++	++++	++++	++++
0.50	0	+	++	++++	++++	++++	++++	++++
0.25	0	++	++++	++++	++++	++++	++++	++++
0.12	0	+++	++++	++++	++++	++++	++++	++++
0.06	++	++++	++++	++++	++++	++++	++++	++++
0.03	++++	++++	++++	++++	++++	++++	++++	++++
0.00	++++	++++	++++	++++	++++	++++	++++	++++

* It should be noted that on all of the media employed growth of this resistant strain was considerably less than that of the susceptible strain.

† Amount of growth after incubation for 21 days estimated by visual examination and graded from 0 (no growth) to ++++ (growth equal to that on medium containing an antimicrobial drug). The inoculum was 0.025 ml. of a 1:1,000 dilution of a fully grown dispersed culture in liquid medium.

concentration of isoniazid required for significant inhibition in this medium was still roughly four times that needed in oleic-acid–albumin agar. Higher concentrations of semicarbazide, which might well have resulted in the liberation of additional isoniazid from the charcoal, were unfortunately bacteriostatic.

The data in table 3 dealing with the moderately resistant strain RD illustrate that one may readily differentiate, using charcoal agar containing semicarbazide, between such a moderately resistant strain (end point at 2 γ of isoniazid per ml.) and a susceptible strain of *M. tuberculosis*.

It was also noted that the end point in charcoal agar medium was often diffuse, a gradual diminution in growth resulting from increasing concentrations of drug. Furthermore, as mentioned above, the charcoal agar containing semicarbazide still inactivated isoniazid to some extent. These two undesirable features made it difficult to detect on this medium strains of tubercle bacilli only mildly resistant to isoniazid (less than 1 γ per ml.).

In summary: Charcoal agar medium was found to be satisfactory for determination of the susceptibility of tubercle bacilli to streptomycin and to PAS. However, the antibacterial activity of isoniazid was markedly diminished in this medium, apparently because of adsorption of isoniazid to the charcoal. Semicarbazide was also found to be adsorbed by charcoal and, when this compound was added to charcoal agar medium, isoniazid activity in this medium was increased. Even the charcoal agar medium containing semicarbazide was, however, not entirely satisfactory for isoniazid-susceptibility determinations.

Marked or moderate isoniazid resistance (greater than 1 γ per ml.) could be detected on such a medium, but it was difficult to differentiate reliably between mildly resistant (less than 1 γ per ml.) and susceptible mycobacteria.

Laboratories and Hospital
The Rockefeller Institute
for Medical Research
New York 21, New York

Edward Lewin[a]
James G. Hirsch

November 24, 1954

[a] Present address: Washington University School of Medicine, St. Louis, Missouri.

COMPARISON OF ROENTGENOGRAPHIC AND SURGICAL FINDINGS IN TUBERCULOSIS

Multiple-drug therapy and surgical resection of residual lesions are being increasingly used as treatment for tuberculosis. The employment of the resection is placing more responsibility on the radiologist for accurate interpretation of the preoperative lung pathology. The ability of chemotherapy to clear extensive lesions, leaving no disease which warrants resection, poses a challenge to the radiologist. Can he predict exactly what is present and therefore prevent surgery in those cases in which chemotherapy has already satisfactorily modified the lesions? In an attempt to answer this question, 165 cases of patients who had been operated upon were analyzed with reference to the preoperative and operative evaluations of the nature of the lesions. One hundred and seven of the patients were from the Muirdale Sanatorium and 58 from the Veterans Hospital, Waukesha, Wisconsin.

The roentgenograms which were found to be essential for good predictions were the postero-anterior, oblique, and lateral roentgenograms, and the lateral planigrams. Postero-anterior full chest planigrams were also included if no residual disease was seen on the other views. The lateral planigrams were valuable for prediction of distribution of residual lesions. The planigrams were overexposed and were viewed with a strong spotlight.

The cases presented below illustrate several points. The first two cases are examples of disease which responded completely to chemotherapy. In these cases, surgery would not have been done if more weight had been placed on the roentgenographic findings. The other cases illustrate the value of heavily exposed lateral planigrams in predicting the character of residual disease.

The first two cases are selected as representative of the patients who responded to chemotherapy and had insignificant disease predicted on roentgenography and confirmed at surgery.

Case 1. The patient was a female, 23 years old, who developed tuberculosis in October, 1951. Tubercle bacilli were cultured from the sputum in January, 1952. All subsequent sputum cultures were negative for tubercle bacilli. The patient received streptomycin-PAS therapy from December 24, 1951, to April 26, 1953. Thoracotomy was performed on December 16, 1952, and showed minimal fibrosis in the lingula. No resection was considered necessary.

The roentgenogram reproduced in figure 1 shows soft, nodular disease in the lower half of the left lung, which showed complete clearing on all special films. In figure 2, the postero-anterior view shows the clearing which occurred.

Case 2. The patient, a 38-year-old male, developed tuberculosis in September, 1948. Right phreniclasia was performed on May 17, 1949. Pneumothorax was given for seven months in 1951. The patient left the hospital against medical advice in 1951 and 1952. He was readmitted in February, 1953, for reactivation of disease. All sputum and gastric cultures were negative for tubercle bacilli on all admissions. Streptomycin-PAS therapy (the streptomycin twice weekly) was given from February to November, 1953. Thoracotomy was performed in November, 1953, and revealed scarring in the apical segment and a 5 mm. subpleural, calcified nodule on the posterior segment. This had the appearance of a lymph node and was not resected.

In figure 3, a roentgenogram obtained during the last hospital admission is reproduced. The mottled disease, which has spread from that observed on previous films, may be seen. In figure 4, the film shows the complete clearing of the disease which followed chemotherapy; a calcified lymph node may be seen over the second anterior rib.

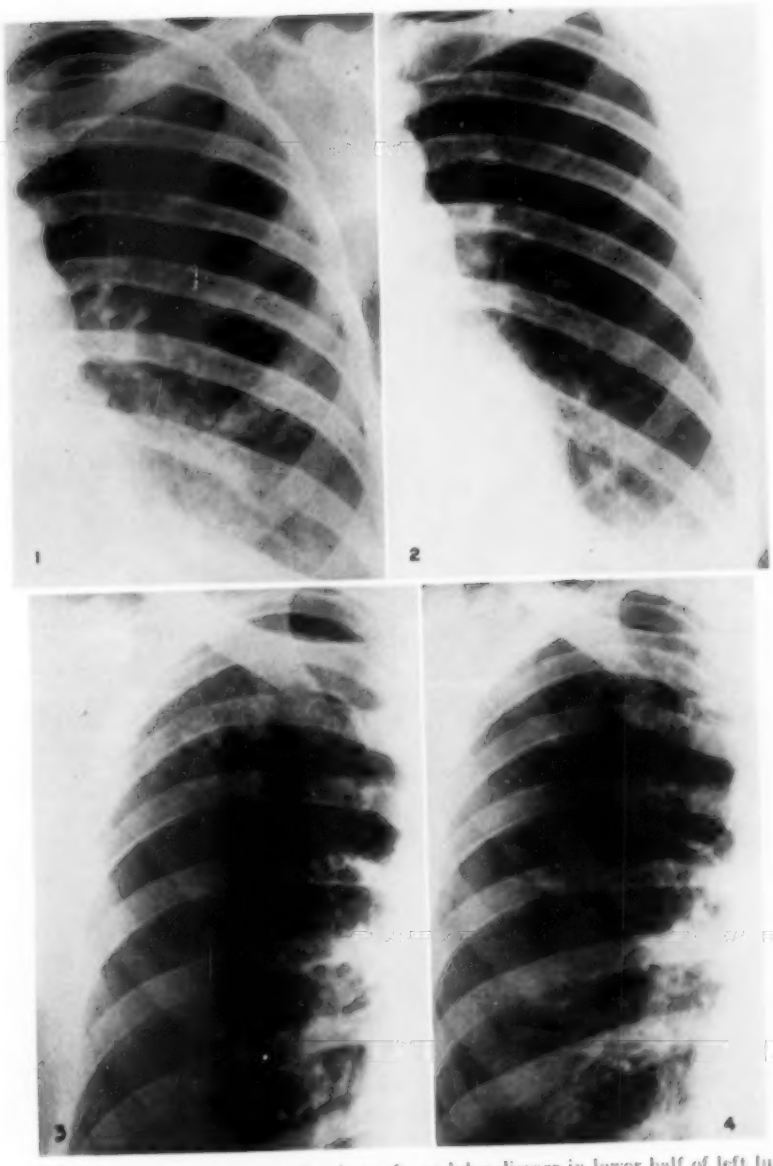


FIG. 1. *Case 1.* Roentgenogram showing soft, nodular disease in lower half of left lung.

FIG. 2. *Case 1.* Postero-anterior view of left lung showing clearing.

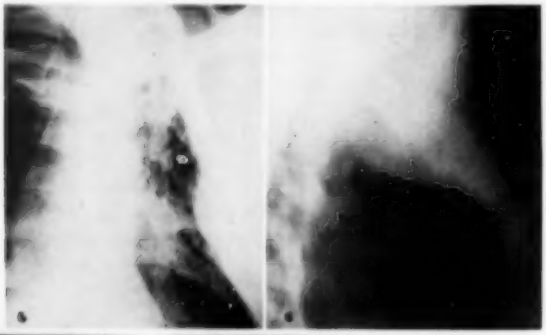
FIG. 3. *Case 2.* Roentgenogram obtained during last hospital admission. Mottled disease, which has spread from that seen on previous films, may be noted.

FIG. 4. *Case 2.* Roentgenogram showing complete clearing of the disease which followed chemotherapy; a calcified lymph node may be seen over the second anterior rib.

The following 3 cases illustrate the value of lateral planigrams:

Case 3. The patient, a male aged 43 years, developed tuberculosis in 1946. A seven-rib, left thoracoplasty was completed on October 6, 1949. Sputum cultures became negative for tubercle bacilli and the patient was discharged on December 18, 1950. Follow-up examination showed a gastric culture positive for tubercle bacilli on April 30, 1953. The patient was readmitted and given isoniazid-streptomycin from June 10, 1953 to the present time. A left upper lobectomy was performed on January 7, 1954. In figure 5, a postero-anterior film of the left upper lobe shows the disease obscured by regenerated ribs. In figure 6 there is excellent visualization of three cavities on one section. The cavities were seen only on lateral planigrams.

Case 4. The patient, a 22-year-old male, developed tuberculosis in June, 1953. Gastric culture was positive for tubercle bacilli in June of 1953. Streptomycin-PAS was ad-



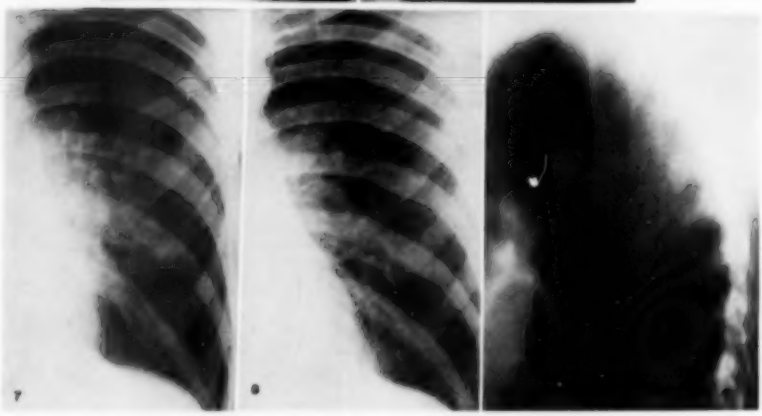


FIG. 5. *Case 3.* Postero-anterior film of left upper lobe showing disease obscured by regenerated ribs.

FIG. 6. *Case 3.* Planigram showing three cavities in one section.

FIG. 7. *Case 4.* Roentgenogram showing soft, patchy infiltration in superior and posterior basal segments of the left lower lobe.

FIG. 8. *Case 4.* Postero-anterior view of left lung showing no evidence of residual disease.

FIG. 9. *Case 4.* Lateral planigram, showing 1 cm. nodule in superior segment of the lower lobe.

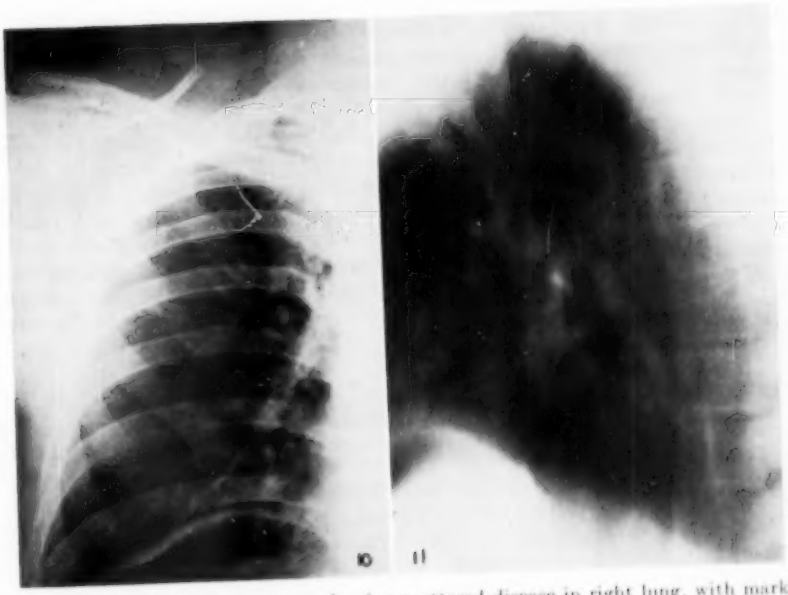


FIG. 10. *Case 5*. Roentgenogram showing scattered disease in right lung, with marked infiltration in apex and first anterior interspace.

FIG. 11. *Case 5*. Planigram showing scattered disease, with marked bronchiectasis in contracted apical and anterior segments.

ministered from June, 1953, to the present time. The patient was offered thoracotomy but has refused the procedure. In figure 7, a soft, patchy infiltration in the superior and posterior basal segments of the left lower lobe may be seen. The patient also had right-sided disease which has completely cleared.

In figure 8, a postero-anterior view of the left lung shows no evidence of residual disease. In figure 9, a lateral planigram, a 1 cm. nodule is shown in the superior segment of the lower lobe. This lesion is not seen on other views because of proximity to the dorsal spine.

Case 5. The patient, a 24-year-old male, developed tuberculosis in November, 1951. Gastric culture was positive for tubercle bacilli in December, 1951. The patient was treated with isoniazid-streptomycin from April 19, 1952 to the present. A minimum resection of at least the entire upper lobe was considered necessary on the basis of the preoperative films. This impression was confirmed at surgery, and an upper lobe lobectomy was done, leaving small nodules in the middle and lower lobes.

The scattered disease in the right lung is shown in figure 10, with marked infiltration in the apex and first anterior interspace. Scattered disease is shown on the lateral planigram (figure 11), with marked bronchiectasis in contracted apical and anterior segments.

The most important prediction made from the preoperative roentgenographic findings is the distribution of the disease. In the comparisons analyzed, a correct prediction was made in 60 per cent of the cases. A minor error related to position of the suture line was found in 22 per cent. There was a gross error of 18 per cent.

The failures in predicting distribution are of concern. In analyzing them, it was found

that they occurred most frequently in extensive disseminated disease. The difficult areas to evaluate were the anterior upper lobe segments and posterior basal segments. More planigrams in questionable cases would undoubtedly have helped in evaluating the presence or character of disease in these areas.

Once the distribution of the disease is determined, it is fairly easy to predict the extent of the excision which will be necessary. The percentages of error on the extent of surgery were identical to those found in predicting distribution. The gross error of 18 per cent included all cases in which it was necessary to remove a subsegment or more than the amount of tissue predicted at the preoperative evaluation.

Cavities were found to be present as predicted in 38 per cent of the patients. Moreover, no cavities were found at surgery which had not been predicted on preoperative roentgenography. In 8 per cent of the cases, however, cavities were thought to have been present on the basis of roentgenography, and no cavities were found at the time of surgery.

As a point of additional interest, an attempt was made to predict the pathologic character of the lesions for which surgery was performed. Caseation was predicted and was found in 72 per cent of the cases. The presence of caseation was predicted and was not present in 2 per cent. In 7 per cent of the cases, caseation was found, but its presence had not been predicted.

The presence of fibrocalcareous nodules was predicted and was found in 19 per cent of cases and was predicted and not found in 2 per cent. In 14 of this group the disease present was predicted to be insignificant, and this was found to be so in 12. In this group, however, 1 mm. to 5 mm. nodular lesions were included which were thought to be not worthy of resection, and the surgeon agreed or took a specimen for biopsy. Of these 14 patients, 5 had minimal fibrosis and pleural adhesions with no palpable nodular disease. The 8 others had small nodules as predicted, and all had cultures negative for tubercle bacilli. In 2 patients the lesions were positive for acid-fast bacilli on microscopy; the remaining lesions were negative for acid-fast bacilli on microscopy.

In analyzing the figures, it was found that, of 165 cases, surgery might have been prevented in 12 patients (7 per cent), all of whom had insignificant disease. On the other hand, in 2 of these patients' with insignificant disease, small caseous lesions positive for acid-fast bacilli on microscopy were present. In 2 other patients with previous pneumothorax, caseous lesions were thought to be present, but only adhesions with no palpable nodular disease were found on exploration.

The remainder of the 149 cases were those in which extensive nodular disease was thought to be present, and this diagnosis was confirmed at surgery.

In summary: The findings of this study show that, if cavity is present, preoperative roentgenograms can be relied on to predict its presence but that, in 8 per cent of the cases, the presence of cavity was predicted and not found. The analysis further shows that the roentgenographic interpretation can be relied on to prevent thoracotomy in cases in which only small, insignificant lesions remain after chemotherapy.

231 West Wisconsin Avenue
Milwaukee 2, Wisconsin

Charles E. Schmidt

April 23, 1954

THE EFFECT OF HI INTENSITY ULTRAVIOLET FOR STERILIZATION

Increased interest in experimental studies pertaining to tuberculosis has led in recent years to a growing need for knowledge concerning methods whereby personnel may be protected from infection and working areas sterilized. In a recent communication[1], a closed sterile area under a negative pressure of approximately one-fourth inch of water was described.

The area is supplied with sterile air by means of a double bank of disposable Air Mat Dry Filters (Type K, manufactured by the Air-gard Manufacturing Co., Chicago, Illinois). Negative pressure is maintained by the use of a blower and the exhaust air is passed over another double bank of the same type of filter as described above. The area is covered by two types of ultraviolet: Hi Intensity ultraviolet[2] for sterilization of the area and standard ultraviolet for use while personnel are operating within the area. Access to the area is gained by the use of an air lock[3] which is similarly covered by both types of ultraviolet lighting. During the actual handling of specimens or microbiologic cultures, *all* ultraviolet lights have been turned off in order to avoid possible death of tubercle bacilli which might be exposed directly to the rays.

This type of sterile area is used routinely for cultural studies with *Mycobacterium tuberculosis*. Animal studies are conducted in similar closed areas differing in that Hi Intensity ultraviolet lighting is utilized only in the air locks through which one gains access to the animal rooms, while standard ultraviolet lights are kept on twenty-four hours daily within the animal rooms themselves.

The present study was conducted in order to evaluate the extent to which Hi Intensity ultraviolet light is capable of sterilizing such working areas.

A six- to seven-day culture of the H37Rv strain of *Mycobacterium tuberculosis* was used throughout. Oleic-acid-albumin agar plates were seeded with 0.1 ml. of culture, and equal numbers of the plates were exposed to Hi Intensity ultraviolet light for varying periods of time. The plates were located at a distance of six feet from the Hi Intensity ultraviolet lights during the period of exposure. Plates were then incubated at 37° C. for a period of twenty-one days, and the number of surface colonies apparent at this time period was counted. In all instances, plates simultaneously seeded with *Mycobacterium tuberculosis* but *not* exposed to Hi Intensity ultraviolet light prior to incubation served as a basis for comparison.

The results obtained in three separate experiments are illustrated in table 1[4]. Plates inoculated with large numbers of organisms were sterilized by exposure for fifteen hours to Hi Intensity ultraviolet light, while a significant decrease in the number of viable cells occurred within one-half hour to three hours.

The experiments described herein were conducted within the environmental conditions of a closed sterile area. The sterilizing capacity of the Hi Intensity ultraviolet light was measured in terms of its ability to kill actively metabolizing cells located on the surface of an oleic-acid–albumin–agar medium. Even under such optimal conditions, a long period of exposure was necessary to bring about sterilization. Cells protected from the

[1] Hobby, G. L., *et al.*: Am. Rev. Tubere., 1954, *70*, 191.

[2] Fifty-two watts are used to cover this area, which has an air space equivalent to 1,098 cubic feet. Within this area is a sterile hood with an air space equivalent to 24 cubic feet and covered by 19 watts of ultraviolet light. Homogenization of infected tissues and similar procedures are carried out. Within the hood, routine procedures are performed in the larger area.

[3] A total of 16 watts is used to cover this area, which has an air space equivalent to 178 cubic feet.

[4] Fenner, F., *et al.*: Ann. New York Acad. Sc., 1949, *52*, 751.

TABLE 1

THE STERILIZING CAPACITY OF HI INTENSITY ULTRAVIOLET LIGHT
(*Mycobacterium tuberculosis:* H37Rv)

Experiment Number	Number of Viable Cell Units* per Plate Prior to Exposure	Time of Exposure	Number of Colonies† per Plate After Exposure				
			Plate Number				
			1	2	3	4	5
1	320,000	0	++++	++++	++++	++++	++++
		10 seconds	++++	++++	++++	++++	++++
		60 seconds	++++	++++	++++	++++	++++
		5 minutes	+++	+++	+++	+++	+++
		30 minutes	+	1	2	1	7
		60 minutes	0	1	2	2	1
2	1,240,000	0	++++	++++	++++	++++	++++
		30 minutes	1(+)	3(+)	1	0	3
		60 minutes	1	1(+)	1(+)	1(+)	8(+)
		2 hours	0	0(+)	0(+)	1(+)	0(+)
		3 hours	2	0(+)	0	0	0(+)
3	1,107,500	0	++++	++++	++++	++++	++++
		15 hours	0	0	0	0	0

* Number of viable cell units was estimated by the method of Fenner, Martin, and Pierce.[4]

† The amount of growth on plates showing more than 100 colonies was measured according to the scale +, ++, +++, ++++. The number of colonies per plate was counted accurately in all other instances. The number of colonies in the central exposed portion of the plate is indicated by the numerical figure; while (+) indicates that heavier growth was present near the rim of the plate.

direct rays by the glass rim of the plate, in particular, showed a tendency to survive during prolonged periods of exposure. These observations with Hi Intensity ultraviolet light make it seem probable that sterilization is seldom achieved with the standard ultraviolet lights so widely used in laboratories in which pathogenic microorganisms, such as *M. tuberculosis*, are handled.

It must be recognized that the inoculum used in the present experiments was large, and only rarely would contamination of a laboratory area with so many microorganisms occur. Smaller numbers of cells undoubtedly would be rendered nonviable in shorter periods of time. Nevertheless, the fact that contaminating organisms are not always located on open surfaces implies also that at times they may not be reached by the ultraviolet rays. To assume, therefore, that sterilization has been accomplished may lead to a false sense of security.

Laboratory accidents unquestionably occur in a matter of seconds, and no device such as an ultraviolet lamp can protect laboratory personnel from contamination at such times. The use of Hi Intensity ultraviolet light, along with other protective measures, however, may prevent the propagation of infectious agents within the laboratory if employed for adequate periods of time.

CHAS. PFIZER & CO., INC.
630 FLUSHING AVENUE
BROOKLYN 6, NEW YORK

GLADYS L. HOBBY
TULITA F. LENERT

November 15, 1954.

NOTE ON THE SECONDARY FACTORS INVOLVED IN THE ETIOLOGY OF SARCOIDOSIS

The recent note of Carr and Gage has led to the summarization of a series of answers to inquiries regarding the geographic distribution of sarcoidosis mailed to most of the medical schools outside the Iron Curtain. In addition, inquiries were also mailed to the World Health Organization and from their central office in Geneva to a large number of their field physicians. It is proper to acknowledge the considerable aid offered by the British Ministry of Health, Canadian Tuberculosis Association, Canadian Ministry of Health, and the Belgian Ministry of Colonies. All of these governmental and international organizations were of great assistance in obtaining a wider distribution of inquiry.

Among factors which might be involved in the etiology of sarcoidosis are those relating to age, sex, race, and geography. The incidence of the disease apparently is just about equal in the two sexes. The literature contains repeated references to the fact that sarcoidosis apparently is quite rare in persons less than ten years old and gradually increases in frequency up to the decade between twenty and thirty years, at which it seems to have its highest incidence.

Particular reference was made in these inquiries to the subject of the race of the persons who acquired the disease. This interest arose from the fact that this writer, in the course of fairly considerable experience in several hospitals in this area, had failed to encounter a single case among Spanish Americans. It may be stated that the majority of these people are of Mexican-Indian descent, and the percentage of Indian or European ancestry may vary to a considerable extent. The obvious suggestion was that in some way the American Indian might be protected by habit or environment from sarcoidosis. This inference led to considerable correspondence with the U. S. Public Health Service and with various medical officers stationed at Indian hospitals. To summarize briefly, Aronson replied that, in his extensive roentgenographic surveys, he has never encountered a single instance among Indians. Likewise, Dahlstrom of the Indian Hospital Service in New Mexico has never seen sarcoidosis in his large experience, and E. J. Johnson, during many years of observation, has encountered no sarcoidosis among Indians of the Northwest. In Oklahoma City, Lamb, with a large and long dermatologic experience, which included many Indians, never encountered a single case of sarcoidosis among them. On the other hand, Harrell has reported a single case in his series from North Carolina; and Haitt, in the same state, encountered one case (whether or not these could be the same patient is not evident nor is it clear whether these patients might have admixtures of Negro or European blood).

From Canada, Falconer, with a wide experience in Alberta, Yukon, and the Northwest Territory, has never encountered sarcoidosis; nor has Brown from a smaller study among the natives of the Canadian Eastern Arctic. Brien states that, while in Ontario there are fairly numerous cases among whites, he has seen none among Indians. From Manitoba, Elliott writes that, although he sees a number of white patients with sarcoidosis, he has seen only one case in an Indian, who was a half-breed. It would thus appear that so far as available information is concerned, sarcoidosis is a disease which is considerably less frequent in Indians than in whites. The only instances thus far discovered consist of the Canadian half-breed and the two Indians from North Carolina.

Since it is common experience that sarcoidosis is much more frequent among Negroes in the United States than among whites, it seemed worth while to inquire of the various colonial services and possessions with stations in Africa about the incidence of sarcoidosis among the natives. Unfortunately, the information obtained is very spotty, and ad-

mittedly probably inaccurate, owing to the natives' fear of physicians, the remoteness of villages, and perhaps to some extent the lack of enthusiasm among medical officers.

From the Belgian Congo, Duren, the inspector-general of hygiene, writes that there is not a single case on record in his files of pathologic reports and that he has encountered no reports of the disease in any files to which he has had access. Silvera of Nigeria reports not a single histologic picture compatible with sarcoidosis in the small files of the University College of Ibadan. From Johannesburg, however, Becker of the Institute of Medical Research writes that sarcoidosis is now and again encountered among natives of that region. It may be remarked that a great many of the African natives in the Union of South Africa are living under conditions partly imposed by the white man. In view of the scanty information, it is necessary to draw conclusions with extreme care, but it does appear that sarcoidosis may not be quite so frequent among Negroes of Africa under native conditions as among Negroes in this country.

In regard to the yellow races, recent events in China have made it impossible to obtain information, except of a very peripheral and highly tentative type. From Taiwan comes information that there is not a single case on record in any institution on the island. Oblath, in review of cases in the hospital and clinics in the University of California, reported no Chinese cases discovered in San Francisco up to 1949, although one case of sarcoidosis in a Japanese had been seen. It is interesting in this regard that, from Singapore, Malaya, Ransome writes " . . .approximately 600,000 pictures (miniature radiographs) have been taken. In addition the various hospitals have been on a look-out for it but so far have not found it. The population of Singapore is approximately 1,000,000, 70 per cent Chinese, 15 per cent Indians, 10 per cent Malays and Eurasians, and 5 per cent Europeans." Vickers also confirms these reports from Singapore, but from Japan come fairly numerous reports, chiefly of the cutaneous or subcutaneous variety (Kitamura, University of Tokyo; Tsusaki, Yokohama). From this information, one might tentatively come to the conclusion that Chinese are, like the American Indians, to a very large degree exempt from the disease.

Geographic distribution: If one makes the assumption that there may be some feature about life in the Scandinavian countries which causes sarcoidosis to attack their inhabitants more commonly than those of other areas, it becomes interesting to know something of the frequency of the disease in other countries lying in approximately the same latitude (56 to 70 degrees north). Portions of Canada lie within these limits, and sarcoidosis among Canadians of European descent is not rare. In Greenland, which lies between 60 and 85 degrees, according to Brøchner-Mortensen, not a single case has ever been discovered, although an extensive roentgenographic survey was carried out there in 1951. Likewise, in Iceland (63 to 67 degrees north) Hjaltested reports that, in fifteen years at the Health Center at Reykjavik, which deals with 60 to 70 per cent of the entire population, he has encountered only one proved case and one doubtful one. All the population of Iceland, he states, is of Norwegian and Irish descent from a migration which took place more than a thousand years ago. He adds, "My opinion is that sarcoidosis is extremely rare in my country compared with what I have seen in most of the other Scandinavian countries and in the U.S.A." Obviously no information is available from the tremendous area of Asiatic and European Russia that lies within these latitudes.

If the other extreme is now considered, those countries which lie within a few degrees of the equator, it should be possible to obtain a fairly clear picture of the total distribution of the disease in terms of geography. The medical schools and the departments of health from which replies have been received are situated in the following countries which lie between 10 degrees north and 10 degrees south; Ceylon, Borneo, Malaya, Indonesia, Sarawak, Nigeria, and Veneguela. From all of these areas, there is the report of but a

single case which was suspected from the roentgenogram but was not studied further. It must be recognized that the total number of persons living within these limits are fewer perhaps than those in the northern extremes, and certainly it is true that public health programs have probably not reached the same level as programs in the north. That these negative findings have some validity, however, is indicated by the following comments: Salfeder at Merida, Venezuela, states that their pathologic sections for two years failed to reveal a single case, and the same type of report comes from Mendez at Caracas. Experience of Ransome at Singapore has already been related. Tjokronegoro of the University of Medicine at Djakarta writes that, among 58,400 surgical specimens and 8,900 post-mortem examinations performed at their school since 1927, there has not been a single case of sarcoidosis.

South of the equator, in the more temperate zones, fairly numerous reports have come from Argentina, New Zealand, and Australia. Both letters and reports from these areas, intermediate between the extremes of north and south, suggest an intermediate frequency between that of the Scandinavian countries and that of equatorial areas.

The possibility that certain foods or their absence might have a bearing on the problem led to inquiry in regard to the religion of patients in those areas in which the disease was less common. Since it was impossible to ask in detail about habits of particular individuals, it seemed possible that food taboos of various religious groups might offer a hint to possible lines of study. From this standpoint, the replies from the Near East are particularly interesting. Laufer, from the Department of Pathology at Jerusalem, states that in eight years they encountered four tissues interpreted as sarcoidosis, three in Jews and one in an Arab. From the French School of Medicine at Bayreuth comes the statement that in five years thirteen cases were encountered, of which one occurred in an Armenian and the others in Arabs. Reimann, from the American University at Bayreuth, states that one Arab was diagnosed as having the disease. The Arabians at the French school may well have been largely Christian, as possibly was the Armenian.

In Afghanistan, which is largely Moslem and is situated near the same latitudes, four cases are reported from Ali Abad. In India, on the other hand, partly Buddhist, partly Hindu, partly Moslem, the only two cases encountered were both in Hindus; one from Agra, the other from Bombay. Finally, from Viet-Nam, which is chiefly Buddhist but slightly south of the other latitudes, there is not an example of sarcoid tissue in the files of the Department of Pathology at Saigon, which reviewed the period from 1947 to 1953. It thus appears that none of the known food taboos expected from regular adherents of any of these religions would serve to point a line of inquiry.

In summary: Extremely tentative conclusions indicate that American Indians and Chinese are relatively free from sarcoidosis. Possibly it is true that the Negro in his natural habitat is also exempt from sarcoidosis and only develops the disease when he comes in contact with European modes of life. Sarcoidosis, finally, may for the present be considered a disease of temperate and subarctic climates and a comparatively rare one in equatorial areas. It should be emphasized that these are extremely tentative conclusions and that they should be subject to revision as better information becomes available.

John S. Chapman

Department of Internal Medicine
The University of Texas Southwestern
Medical School
2211 Oak Lawn Avenue
Dallas 19, Texas

December 6, 1954

Books

SURGERY OF THE CHEST. A Handbook of Operative Surgery. By Julian Johnson and Charles K. Kirby. Pp. 387, The Year Book Publishers Inc., Chicago, Illinois, 1952, Price, $9.00.

The writers have succeeded in condensing within the confines of less than four hundred pages a detailed presentation of the operations which have been demonstrated to be important in contemporary thoracic surgery.

After an admittedly brief orientation concerning the diseases for which a given procedure is employed, the operative details are described on a left hand page and the appropriate illustrations are plated on the opposite (right hand) page. The plates, more than eighty in number, are principally line drawings, but a minority are half-tones. The former are more successful and many are of superb quality. Deserving of particular commendation are the textual descriptions and plates concerned with segmental pulmonary resection (Chapter 8), the surgery of the esophagus (Chapter 10), and the surgery of the heart and great vessels (Chapter 11). An excellent description is offered of the possible routes to be employed in thoracotomy and their special indications.

One chapter only, Chapter 12, comprising thirty-one pages is devoted to the subject of surgery in pulmonary tuberculosis, exclusive of resection. This seems unduly brief but probably reflects rather accurately the present modes of practice.

This book has already had a wide reception, and the reviewer's copy is from the second printing.

G. E. LINDSKOG
New Haven, Connecticut

LUNG CANCER. By Seymour M. Farber. Pp. 162, with illustrations, Charles C Thomas, Springfield, Illinois, 1954, Price, $4.75.

This monograph is designed as a concise presentation of the subject of pulmonary carcinoma, directed to the general practitioner, who has the initial opportunity to diagnose this disease. The magnitude of the problem presented by lung cancer demands that strong efforts be made to acquaint all segments of the profession with the means to diagnose and combat this increasingly common tumor.

The book succeeds in achieving completeness without prolixity; in 136 pages and a bibliography of 134 items, there is a well-rounded survey of the subject. Directed as it is to "practical" men, it nonetheless recognizes the importance of basic information on the problem and devotes more than 50 pages to etiology and histopathology. This is considerably more than the space allotted to "Clinical Features" and "Surgery." The limitations of surgical treatment are largely set by the nature of the disease, but increased discussion of the clinical features to raise the physician's index of suspicion might be a profitable addition.

In the section on "Surgery," many will disagree that closure of the bronchus "is now accomplished by ligature one centimeter or so above the bronchial stump" relying on "fibrinous clotting in the proximal end" plus a pleural flap to give a secure closure. One might better emphasize the avoidance of trauma, devascularization, and a blind bronchial pouch as fundamental principles in this important technical step. Moreover, the section on lobectomy versus pneumonectomy omits a discussion of lymph node dissection, which may be more thorough in some lobectomies than in many pneumonectomies. Hearty agreement must be given to the author's view that not every "palliative" pneumonectomy

is in the best interests of the patient. On the other hand, the repeated emphasis on elaborate efforts to determine inoperability may lead to denial of operation in some patients susceptible to genuine palliation or even cure.

Throughout the book the author emphasizes the predominant role of those to whom the book is mainly directed, namely, the general practitioner or physician. Many surgeons may take exception to the statement (page 113) that they "... cannot be expected to have the time to establish the personal relationship with the patient that is so important a part of the physician's services ..." and others may be puzzled by the assertion (page 120) that "... (the physician) ... is the only one in medicine who can be expected to give due weight to that inestimable factor, the personality of the patient."

The book is handsomely set up with a minimum of proofreading errors. Some of these occur in the first paragraph on Angiocardiography (page 85), where the intravenous contrast substance is said to be vinyl acetate! One hopes that the high cost of producing such a book will not detract from its wide circulation.

C. Rollins Hanlon
St. Louis, Missouri

BOOKS RECEIVED

Fundamentals of Internal Medicine. Fourth Edition. By Wallace Mason Yater, with the assistance of William Francis Oliver. Pp. 1,276, with illustrations, Appleton-Century-Crofts, Inc., New York, 1954, Price, $13.50

Legal Medicine, Pathology and Toxicology. Second Edition. By Thomas A. Gonzales, Morgan Vance, Milton Halpern, and Charles J. Umberger. Pp. 1,349, with illustrations, Appleton-Century-Crofts, Inc., New York, 1954, Price, $22.00

Tuberculosis in Ireland. Report of the National Tuberculosis Survey (1950–1953). Pp. 301, Medical Research Council of Ireland, Dublin, 1954.

La Vaccination par le B.C.G. By R. Griesbach. Pp. 356, Les Editions Medicales Flammarion, Paris, 1954.

Lehrbuch der Tuberkulose des Kindes und des Jugendlichen. By Werner Catel. Pp. 501, with illustrations, Georg Thieme Verlag, Stuttgart, 1954, Price, $15.70.

El Origin del Bacilo de Koch. By C. Xalabarder. Pp. 140, with illustrations, Instituto Antituberculosis "Francisco Moragas," Barcelona, 1954.

The Brompton Hospital. The Story of a Great Adventure. By Maurice Davidson and F. G. Rouvray. Pp. 151, Lloyd-Luke Ltd., London, 1954.

American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

AMERICAN BOARD OF INTERNAL MEDICINE SUB-SPECIALTY OF PULMONARY DISEASES CERTIFICATION

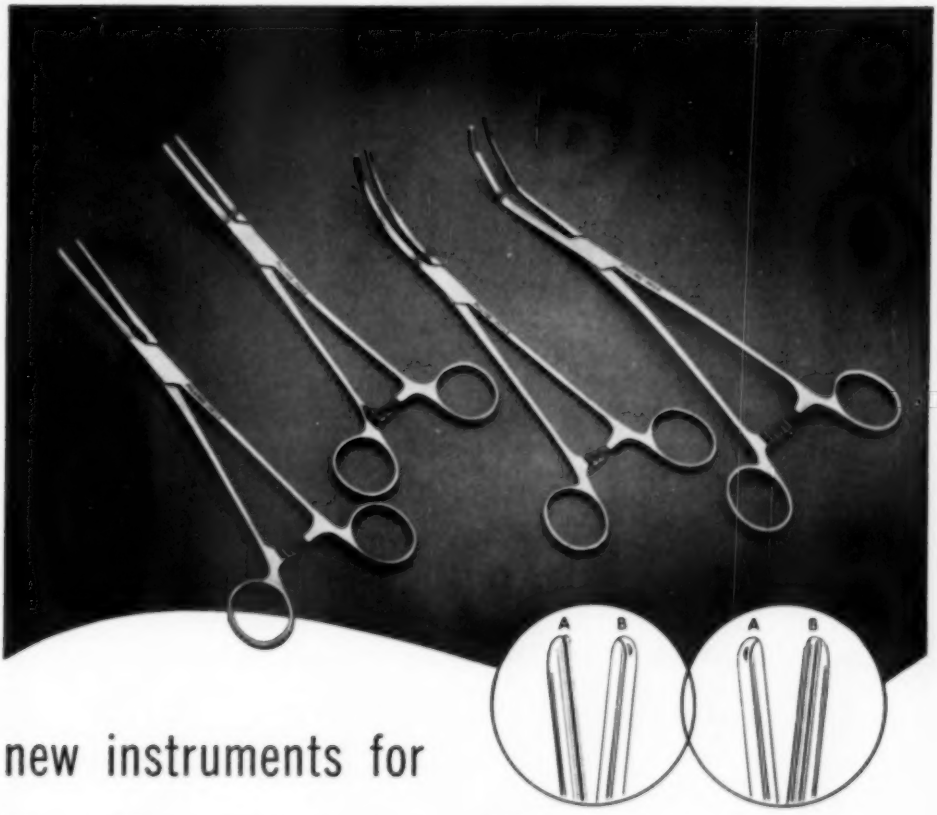
We are writing on behalf of the American Board Sub-specialty of Pulmonary Diseases, about which there seem to be several misconceptions. A few comments might be helpful to the many physicians who specialize in this field but who have never tried for certification.

Everyone is eligible to take the examination who has been certified by the American Board of Internal Medicine, of which "Pulmonary Diseases" is a subsection, who has had one or more year's specialized training in the field, and who specialized at least part time therein.

The examinations are oral and consist of a case analysis and a fairly searching X-ray and clinical quiz. They are scheduled at least twice a year, usually in the East, Midwest, and West; examinations at the time of the annual meeting of the NTA are planned. Membership in chest specialty groups is neither a bar nor a requisite.

Further information about qualifications, time and location of the examinations, et cetera, may be obtained from the Secretary-Treasurer of the American Board of Internal Medicine, Dr. William Werrell, One West Main Street, Madison 3, Wisconsin.

ROGER S. MITCHELL, M.D., *Chairman*
WILLIAM H. OATWAY, JR., M.D.
EDWARD S. RAY, M.D.
JOHN SKAVLEM, M.D.
DAVID SMITH, M.D.
Sub-specialty Board for Pulmonary Diseases
American Board of Internal Medicine



new instruments for

Cardiac Surgery with non-traumatic ATRAUGRIP jaws*

Now the cardiac surgeon has the working advantages of fine, perfectly balanced Pilling-made instruments plus the new, noncutting, non-traumatic ATRAUGRIP jaws.

Two rows of finely serrated teeth on one blade and one row on the other, each set in a separate parallel plane, provide a grip which will not slip despite high intravascular pressures or intricate operative procedures. The surgeon is assured a firm grasp of tissue or complete occlusion of blood vessels without danger of crushed or perforated vascular walls or damage to intimal linings.

Write Pilling for data on other instruments for cardiac surgery.

GLOVER AURICULAR APPENDAGE CLAMP
Th2089

GLOVER COARCTATION CLAMP
Th2251 straight
Th2256 angular
Th2275 curved

GLOVER PATENT DUCTUS CLAMP
Th2261 straight
Th2266 angular

*Pat. No. 2668538

THE AUTHENTIC INSTRUMENT IS AVAILABLE ONLY FROM PILLING—ORDER DIRECT:

GEORGE P. **Pilling** & SON CO.

3451 WALNUT STREET, PHILADELPHIA

DIFCO

Sensitivity Testing
of
TUBERCLE BACILLI

BACTO-SENSITIVITY DISKS

Sensitivities to the commonly employed therapeutic agents are accurately and readily determined using Bacto-Sensitivity Disks containing three concentrations of Dihydrostreptomycin, Streptomycin, Viomycin, PAS (p-Amino Salicylic Acid) and INH (Isonicotinic Acid Hydrazide). Concentrated specimen or pure cultures are evenly streaked on Bacto-Peizer TB Medium and Sensitivity Disks applied. Zones of inhibition of growth following incubation indicate if an organism is sensitive, moderately sensitive or resistant to the various chemotherapeutic agents.

Peizer TB Medium recommended for sensitivity test procedures is easily and readily prepared from Bacto-Peizer TB Medium Base and Bacto-Peizer TB Medium Enrichment (Peizer and Schecter: Am. J. Clin. Path., 20:682:1950). This is a selective medium suitable for primary culture and simultaneous sensitivity determinations of tubercle bacilli from concentrated specimen.

BACTO-SENSITIVITY TEST MEDIA

Bacto-ATS Medium and Bacto-Lowenstein Medium are available in screw capped tubes with added chemotherapeutic agents. The ATS Medium is prepared according to the formula specified by the American Trudeau Society; the Lowenstein Medium according to the Jensen modification. These media are processed under carefully controlled conditions to give rapid optimal growth of the tubercle bacilli.

Bacto-ATS Sensitivity Media and Bacto-Lowenstein Sensitivity Media are supplied in sets of 12 tubes consisting of 2 tubes of control medium without therapeutic agent and one tube each of the following:

Streptomycin 10 mcg./ml.	INH 5 mcg./ml.
Streptomycin 100 mcg./ml.	Streptomycin 10 mcg. and PAS 100 mcg./ml.
Viomycin 10 mcg./ml.	Streptomycin 100 mcg. and PAS 100 mcg./ml.
Viomycin 100 mcg./ml.	Streptomycin 10 mcg. and INH 1 mcg./ml.
INH 1 mcg./ml.	Streptomycin 100 mcg. and INH 5 mcg./ml.

The above media are also supplied in packages of 12 tubes of any one medium. In addition, for more detailed determinations, ATS Sensitivity Media and Lowenstein Sensitivity Media containing Streptomycin 250 mcg., Viomycin 250 mcg., and INH 0.2 mcg. are available in packages of 12 tubes.

These media are supplied in incubator packages which permit preferred horizontal incubation.

Bulletin No. 141 describing Sensitivity Testing of Tubercle Bacilli is available upon request.

DIFCO LABORATORIES
DETROIT 1, MICHIGAN

WMA

is Speaking for You...

The World Medical Association is the *only* international organization empowered to speak for *you*—before other international organizations in the interest of the practicing physician.

Here's what the World Medical Association does for you:

1. Gives you a voice in the formulation of policies to meet problems of medical care on an international level; represents *your* interest before such governmental or non-medical policy-making organizations as WHO and ILO.

2. Brings you the World Medical Journal; keeps you posted regarding such problems as social security medical programs, international medical law, standards of medical practice and education.

3. Provides you with a means of exchanging information or visiting with member colleagues throughout the world.

4. Brings you a U. S. Committee certificate of membership for display in your office or reception room.

join now . . . with over 700,000 physicians from 46 countries . . . WMA is your only official voice in world medicine.

WMA is approved by the American Medical Association

•Dr. Louis H. Bauer, Secretary-Treasurer
U. S. Committee, Inc., World Medical Association
345 East 46th Street, New York 17, New York

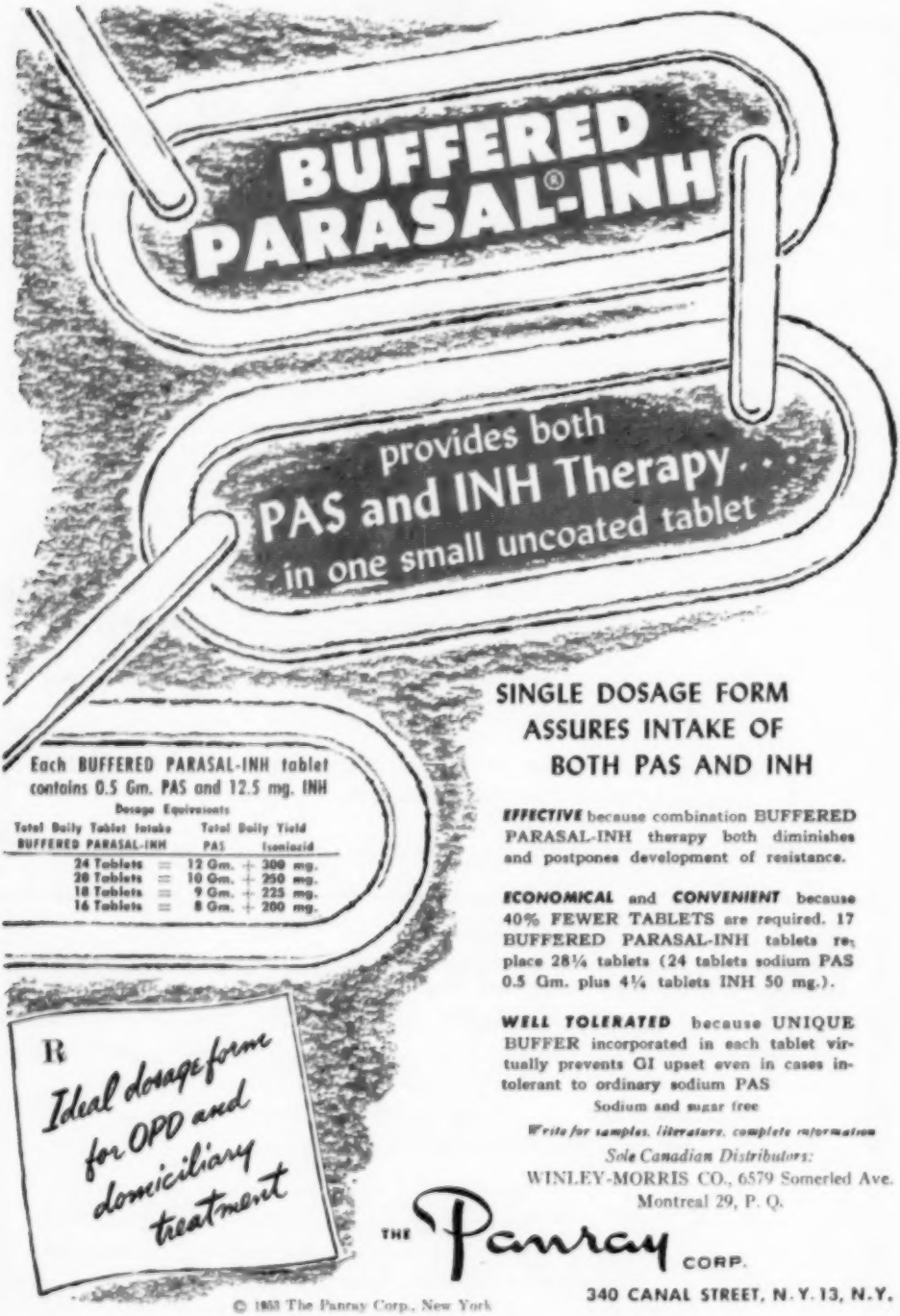
I desire to become an individual member of the World Medical Association, United States Committee, Inc., and enclose a check for $———————, my subscription as a:

———Member —$ 10.00 a year
———Life Member —$500.00 (No further assessments)
———Sponsoring Member—$100.00 or more per year

Signature———————————————
Address———————————————
———————————————

(Contributions are deductible for income tax purposes)
Make checks payable to the U. S. COMMITTEE, WORLD MEDICAL ASSOCIATION

This space donated by the publisher in the interests of the practicing physician



Each BUFFERED PARASAL-INH tablet contains 0.5 Gm. PAS and 12.5 mg. INH

Dosage Equivalents

Total Daily Tablet Intake BUFFERED PARASAL-INH		Total Daily Yield PAS		Isoniazid
24 Tablets	=	12 Gm.	+	300 mg.
20 Tablets	=	10 Gm.	+	250 mg.
18 Tablets	=	9 Gm.	+	225 mg.
16 Tablets	=	8 Gm.	+	200 mg.

℞ *Ideal dosage form for OPD and domiciliary treatment*

SINGLE DOSAGE FORM ASSURES INTAKE OF BOTH PAS AND INH

EFFECTIVE because combination BUFFERED PARASAL-INH therapy both diminishes and postpones development of resistance.

ECONOMICAL and ***CONVENIENT*** because 40% FEWER TABLETS are required. 17 BUFFERED PARASAL-INH tablets replace 28¼ tablets (24 tablets sodium PAS 0.5 Gm. plus 4¼ tablets INH 50 mg.).

WELL TOLERATED because UNIQUE BUFFER incorporated in each tablet virtually prevents GI upset even in cases intolerant to ordinary sodium PAS

Sodium and sugar free

Write for samples, literature, complete information

Sole Canadian Distributors:
WINLEY-MORRIS CO., 6579 Somerled Ave.
Montreal 29, P. Q.

THE Panray CORP.

340 CANAL STREET, N. Y. 13, N. Y.

© 1953 The Panray Corp., New York

NAPT publications

BCG AND VOLE VACCINATION

A Practical Handbook by K. NEVILLE IRVINE, M.A., D.M., BCh., M.R.C.S., L.R.C.P., Adviser in B.C.G. Vaccination to Oxford Regional Hospital Board

Foreword by FREDERICK HEAF, M.A., M.D., F.R.C.P.
David Davies, Professor of Tuberculosis, The Welsh National School of Medicine.
96 pages, comprehensive index, 10 coloured plates, Clothbound three dollars.

CHEMOTHERAPY IN THE TREATMENT OF TUBERCULOSIS

A symposium of 11 articles, based on papers read at a postgraduate course for doctors held by the Tuberculosis Educational Institute at Cambridge in September 1953.

60 pages, diagrams and graphs, 2 pages of illustrations, one dollar

Order direct from

NATIONAL ASSOCIATION FOR THE PREVENTION OF TUBERCULOSIS
Tavistock House North, Tavistock Sq. London, W.C. 1

Now Available in Spanish

Clasificación y Diagnóstico Standard

(DIAGNOSTIC STANDARDS)

First published by the National Tuberculosis Association in 1950 and now in regular use by more than 100,000 physicians, this accepted authority on terminology, standard tests and classification in tuberculosis has now been translated into Spanish by Professor H. Orrego Puelma of the University of Chile Faculty of Medicine, assisted by Doctors Raul F. Vaccarezza, Ignacio Baldo and Armando Alonso, and is available to interested physicians in the form of a 64-page publication.

To cover handling costs, a price of 50 cents (American funds or the equivalent) has been established. Your order is cordially invited and will have immediate attention.

Please address orders to:

DR. H. ORREGO PUELMA

Hospital del Salvador,

58 Vicuna Mackenna, Santiago, Chile.

POSITIONS AVAILABLE AND WANTED

RATE $3.50 per insertion for 25 words or less; additional words—15 cents each. Bold face semi-display $5.00 per insertion for 25 words or less; additional words—20 cents each. Box number—75 cents extra. Employment Bureau—$10.00 per column inch. Remittance should accompany copy and be sent to: American Review of Tuberculosis, 280 Madison Ave., New York 16, N.Y.

POSITION AVAILABLE

RESIDENT PHYSICIAN: 160 bed tuberculosis hospital in pleasant rural setting. Available July 1. All medical and surgical therapy utilized. Salary range $325.00 to $425.00 plus partial maintenance for family. Tuberculosis experience desirable but not essential. California license mandatory. Apply to Medical Director, Stony Brook Retreat, Keene, California.

MEDICAL DIRECTOR: North American Graduate, five years tuberculosis experience, relatively new 100 bed tuberculosis hospital, Madisonville, Kentucky. Salary $10,000., complete maintenance. Apply State Tuberculosis Hospital Commission, New State Office Building, Frankfort, Kentucky.

POSITION WANTED

Field of tuberculosis, any capacity. On staff of large sanatorium since 1938: Chief, Medical Service, forty-one to forty-four; Asst. Supt. since forty-four. Nearing retirement on reaching statutory age limit of seventy. Native American, active, in good health, no dependents. Position and pay secondary. Address Dr. John M. Stanley, Asst. Supt., Glenn Dale, Md.

whenever
cough
therapy
is indicated...

Hycodan

Bitartrate · Dihydrocodeinone Bitartrate

An AMA Council Accepted Cough Preparation

Syrup (5 cc. per teaspoonful), Oral Tablets (5 mg. per tablet)
May be habit-forming. Average adult dose, 5 mg. t.i.d. p.c.

Endo

ENDO PRODUCTS INC.,
Richmond Hill 18, New York